ROOT AND FLOW RATE CONTROL ON BIOGEOCHEMICAL CYCLING IN TWO HORIZONTAL FLOW CONSTRUCTED WETLAND MESOCOSMS

A Thesis

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Master of Science

by

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ABSTRACT

Constructed wetlands serve as both a pollutant sink as well as a possible greenhouse gas source for runoff treatment. Since environmental parameters controlling these biogeochemical cycles can be controlled, it is important to understand how changes in these parameters can affect nutrient and greenhouse gas cycling and concentrations. Thus, we chose to examine the effects of two poorly-understood parameters, the presence of aerenchymal roots and flow rate, by measuring concentrations of several nutrients and gases on two heavily controlled mesocosm constructed wetlands planted with *Schoenoplectus acutus* and maintained at either high flow (20 ml/min) or low flow (10 ml/min). There was no difference in overall nitrate removal efficiency between the two mesocosms. However, our results indicate that increased flow rate was associated with higher oxygen and nitrate concentrations in the porewaters. The presence of aerenchymal roots increased methane and decreased nitrous oxide concentrations as compared with substrate containing no roots. The differences in methane and nitrous oxide patterns may be due to aerenchymal plants competing for N-uptake with microbes and highlights the importance of species diversity and richness in studying the impact of plants on wetland control. The study also demonstrated the key role that subsurface flow rates, and the associated property of hydraulic retention times, can play in the biogeochemical functioning of constructed wetlands.

BIOGRAPHICAL SKETCH

Sanam Anwar was born in Pittsburg, Kansas and raised in Austin, Texas. She received her Bachelor of Arts degree in Biological Sciences with a minor in Geosciences in 2015 from Wellesley College. After a brief stint back in Austin, she decided to come back to the northeast to pursue her Master of Science in Environmental Engineering degree at Cornell University. Through her time at Cornell, she was lucky enough to find a spot in the Dept. of Biological and Environmental Engineering's Soil and Water Lab. After finishing her degree, Sanam decided to work in environmental consulting in her home state of Texas. She hopes to use the technical skills she learned in pursuing this degree along with her liberal arts education to one day pursue a career in environmental diplomacy.

Dedicated to my parents, Israr and Sarwat Anwar, and my grandparents, and to all my mentors throughout my life. I couldn’t have done any of this without you.

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LIST OF ABBREVIATIONS

FAO	Food and Agricultural Organization of the United Nations
GHG	Greenhouse Gas
GHGs	Greenhouse Gases
HRT	Hydraulic Retention Time
IPCC	International Panel on Climate Change
RH	High-flow reactor (20mL/min)
RL	Low-flow reactor (10mL/min)

PREFACE

Constructed wetlands are a cost-friendly and generally fossil fuel-free method of removing pollutants and excess nutrients from a system and serve as an alternative to conventional activated sludge treatment plants. While constructed wetlands may provide an ideal environment for beneficial removal to take place, these wetlands have also been shown to produce harmful greenhouse gases, such as carbon dioxide, methane, and nitrous oxide (Hiraishi et al., 2013). While many of the mechanisms behind carbon dioxide production are well understood, the International Panel on Climate Change of 2014 (IPCC) report on wetlands determined that the mechanisms behind the production of methane and nitrous oxide are very poorly understood(Hiraishi et al., 2013).

Increasing nutrient loading rates due to greater worldwide agricultural fertilizer application and changing climatic conditions throughout the world represent formidable problems now and in the coming years(FAO, 2017; Hiraishi et al., 2013; Horton, Bader, Rosenzweig, DeGaetano, & Solecki, 2014; Salmon-Monviola et al., 2013). Thus, it is important to understand how we can continue to remove excess nutrients, like nitrate, while also mitigating the production of harmful greenhouse gases, like methane, carbon dioxide, and nitrous oxide.

Introduction

The Food and Agricultural Organization of the United Nations (FAO) projects that worldwide application of fertilizer nutrients will increase by 1.4% from 2015 to the end of 2020 (FAO 2017). Much of this fertilizer ends up in agricultural runoff, a major source of nutrient pollution worldwide, which can cause both eutrophication of natural waterways and depletion of dissolved oxygen (Saeed and Sun 2012).

In addition to adapting to this increase in fertilizer application, agricultural runoff treatment must be modified to adapt to the consequences of anthropogenic climate change that have already caused major changes in predictable weather patterns throughout the world. In the northeastern United States, extreme weather events and intense seasonal precipitation have increased by 74% over historical levels, with an average increase of 10mm/decade over the last 50 years (Horton et al. 2014; Guilibert et al. 2015), leading to greater discharge and flooding in freshwater systems throughout the region (R. W. Howarth et al. 2006), and these events are expected to increase. The results of these hydrologic changes include increases in erosion and sediment and nutrient transport, which along with overall warming, are significantly impacting our ecosystems (Program 2017; Horton et al. 2014).Currently, the most widely used treatment for agricultural runoff is the conventional activated sludge treatment plant (Kadlec and Knight, 1996). However, constructed wetlands offer an alternative treatment free from the disadvantages of fossil fuel energy sources and harsh chemicals, making this method both environmentally and economically more attractive than conventional treatment (Kadlec and Knight, 1996). Engineered ecosystems, like constructed wetlands, differ from natural wetlands and are designed to control many environmental parameters that affect biogeochemical cycling and the wetland's ability to treat wastewater.

Wetland scientists are working to determine how various factors influence the environmental parameters controlling nutrient and greenhouse gas cycling from anthropogenic sources. Some of these parameters include presence of aerenchymal plants, temperature, pH, erosion, denitrification, carbon content of soils, as well as flow rate of water through the system stream (Hiraishi et al. 2013; Lee and Scholz 2007; Rysgaard et al. 1994; Stanley and Ward 2010).

While some of these factors are well understood and are also regularly controlled in constructed systems, many other uncertainties remain. These uncertainties may affect the ability of constructed wetlands to adequately remove pollutants and have even more serious consequences, namely the production of greenhouse gases (GHGs) (Hiraishi et al. 2013). With increased nutrient input and more extreme weather events because of climate change, system ineffectiveness may exacerbate GHG production and water quality degradation. Thus, by improving our understanding of these parameters, we can be better prepared for increased loads and extreme weather events.

The International Panel on Climate Change (IPCC) outlines several specific unresolved environmental parameters that affect biogeochemical cycling, including flow rate of wastewater through constructed wetlands and the presence of aerenchymal roots within constructed wetlands (Hiraishi et al. 2013). In this study, we seek to decouple the effects of two competing factors and focus on these parameters.

The IPCC 2013 Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories on Wetlands identified both nutrient loading and flow rate of constructed wetlands as two of the key environmental parameters affecting biogeochemical cycling of wastewater through these systems (Hiraishi et al. 2013). However, the report also identifies the conflicting evidence of the effects of these parameters, giving as examples studies linking increased flow and loading rates to higher greenhouse gas emissions and contradicting studies which recommend increased flow rates as a mitigation strategy for reducing emissions ((Hiraishi et al. 2013).

Theoretically, increased flow rate decreases the amount of bed contact time for wastewater and would thus limit transformation of compounds (Saeed and Sun 2012). However, work by Saeed and Sun (2012) indicate this is not a clear-cut scenario. They suggest that anoxia can cause differential effects depending upon flow rate, as the lack of oxygen would inhibit certain processes while enhancing others, (i.e. nitrification and denitrification respectively) and leads to a difference in microbial environment.

In general, higher oxygen concentrations are correlated with a lower rate of nitrous oxide production, regardless of nitrate input rate (Rosamond, Thuss, and Schiff 2012). Their study, like most other reviews, relies on consolidation of data from drastically different environments, from natural riverine environments to operational constructed wetlands. Many other parameters could have also caused these differential effects, including changes in pH, temperature, macrophyte species richness, or influent concentrations (R. W. Howarth et al. 2006).

Another key unresolved environmental parameter identified by the IPCC supplement is the impact of the presence of aerenchymal roots. Aerenchyma are longitudinally-connected spaces within either roots or shoots of plant tissues that facilitate gas transfer beyond normal levels in plants (Takahashi et al. 2014; Evans 2004). Two major types of aerenchyma are formed by plants, schizogenous and lysigenous aerenchyma. Schizogenous aerenchyma form by differential growth of cells and are characteristic of certain plant species, while lysogenous aerenchyma are formed by cell death and are only formed as a response to stress (Takahashi et al. 2014; Evans 2004). Many species exhibit both forms of aerenchyma formation at different times in their development and as a response to different stressors (Evans

2004). Constructed wetlands are likely habitats for aerenchymal plants as continuous flooding often leads to anoxic stress in these plants.

Presence of aerenchyma is involved in the formation of GHGs from constructed wetlands through the introduction of oxygen from the surface to the subsurface of otherwise hypoxic wetlands (Hiraishi et al. 2013). This excess gas transfer is beneficial for the plant, as it allows for the increased flow of carbon dioxide and oxygen through the plant (Society 2016). However, it may also allow for GHGs, like nitrous oxide and methane that form in the subsurface of nutrient-rich plants, to escape into the atmosphere (Lai et al. 2011; R. Inamori et al. 2007; Henneberger et al. 2017; Jørgensen, et. al. 2012). There is conflicting evidence as to whether aerenchyma are the culprit behind some greenhouse gas emissions. N-uptake by plants may serve as competition for microbial communities that produce nitrous oxide, thus decreasing overall nitrous oxide emissions (Silvan et al. 2005; Ryuhei Inamori et al. 2008). The conflicting evidence may be a result of experiments conducted using ex-situ designs with no control for plant species.

To better understand the relationships between aerenchymal roots, flow rate, and nutrient cycling in constructed wetlands, we examined two monoculture wetlands flowing at two different flow rates with areas of root exclusion. This study aimed to characterize the relationships between groundwater flow rates and the presence of aerenchymal roots with concentrations of porewater nutrients and dissolved gases in constructed, sub-surface flow wetlands. This was accomplished by measuring selected nutrients and dissolved gases within each of two constructed wetland mesocosms under controlled conditions of flow rate and nitrate inputs. The chemical constituents measured are critical in the biogeochemical cycling of nitrogen, an agricultural pollutant, i.e. nitrate, nitrous oxide, oxygen, carbon dioxide and methane. To characterize these relationships, four main questions were asked. Three questions were asked to characterize the relationship between flow rates and biogeochemical cycling in constructed wetlands:

1) Does flow rate influence the mean concentrations of nitrate and dissolved gas in the constructed mesocosms?

2) Does flow rate impact patterns of chemical concentration along the flow paths within the constructed mesocosms?

3) Does a change in subsurface flow rate impact relationships among measured chemical constituents?

An additional question was asked to characterize the relationship between the presence of aerenchymal roots and biogeochemical cycling in constructed wetlands:

4) Does the presence of aerenchymal roots affect differences in nutrient and dissolved gas concentrations within constructed wetlands?

Materials and Methods

2.1. Reactor Setup

Constructed wetlands were modeled as two continuous, horizontal sub-surface flow (HSSF) mesocosm reactors with dimensions 50.8 cm x 25.4 cm x 30.5cm within a clear plastic tub that was covered along the sides with aluminum foil to eliminate light penetration and prevent algal growth (Figure 1). Reactors were kept in the same greenhouse and maintained at a uniform temperature of 24 ºC with 12 hours of sunlight, simulated with overhead artificial lighting, each day. Both reactors were seeded with inoculum soil (10% by volume sourced from shoreline of nearby Beebe Lake, Ithaca, NY), 5% by volume ground leaf litter, and 85% multipurpose sand (Lowe's Hardware Store) with a combined mineral porosity of 31.84% determined via a standard bulk porosity equation, dividing void volume by the total volume.

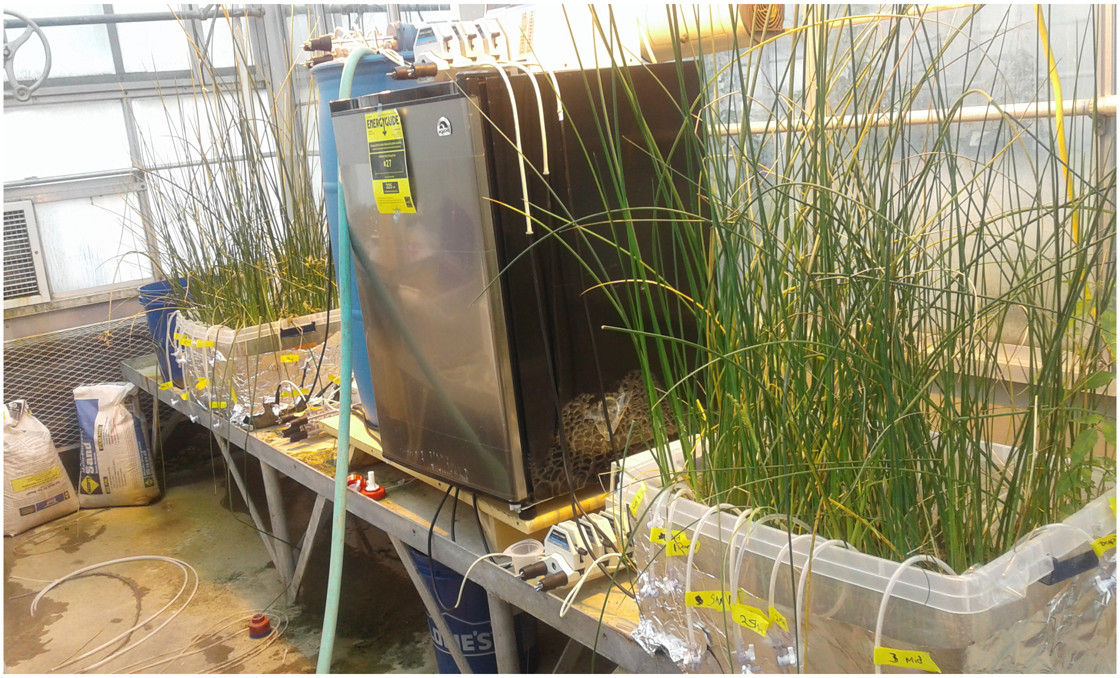

Figure 1. Photo of actual sampling setup in reactors used during experimentation. The Low Flow Reactor (10mL/min) is pictured on the left and the High Flow Reactor (20 mL/min) is pictured on the right. Reactors were maintained with continuous wastewater flow through for one month before experimentation.

The reactors were fed continuously via three evenly-spaced influent ports with tap water containing dissolved nitrate-N (0.2 g/L), phosphorus (0.01 g/L), and also glucose (0.05 g/L) to provide a carbon source (Aiyuk and Verstraete 2004), a mix chosen to simulate agricultural wastewater (Figure 2). The reactors were drained with a single effluent port located in the middle/bottom of each reactor. Influent ports were also installed in the subsurface of the reactor but were 2.5 cm above the effluent port in each reactor. Dissolved oxygen (DO) concentrations of porewater samples met the standard of anaerobic conditions with an average concentration of 0.83mg/L in RH and 0.64mg/L RL, pH was also the same between both reactors at pH=7.6 (EPA, 2016). Flow was initiated immediately after construction and maintained for the entire duration of the experiment.

The flow rate for the Low Flow Reactor (RL) was 10 mL/min, with an average retention time of 20.3 hours and the flow rate for the High Flow Reactor (RH) was 20 mL/min with a retention time of 10.15 hours. These residence times and flow rates were chosen to represent the range found in constructed wetlands designed to reduce influent nitrate (Lucas and Greenway, 2011). Each reactor was planted with young *Scirpus acutus*, also known as *Schoenoplectus acutus*, an aerenchymal plant which forms both schizogenous and lysigenous aerenchyma (Evans, 2004). Twenty bulbs were planted in each reactor in a five by four pattern. Plants were grown for one month before experimentation started. This allowed them to reach maturity and adjust to wetland conditions. At the time of porewater sampling, plant growth was similar between the two reactors, as they grew at the same rate based on a visual monitoring of average height.

Additional details on reactor design and construction, and preliminary equipment calibration are all provided in the Appendix.

2.2 Monitoring Wells

Nine porewater monitoring wells were placed throughout each reactor. Wells were placed at three depths as well as locations in the front, middle, and end of the reactors, representing nine total bulk soil monitoring wells. The wells were considered to represent three replicates at the front, middle and end of the reactor (Figure 2). Each monitoring well consisted of an air stone attached to flexible, clear, 1/8 in plastic tubing to remove pore water samples from the subsurface.

To determine the effect of roots on nutrient and greenhouse gas cycling in these systems, three root-excluded bags were placed at random locations in each reactor. Root-excluded bags were approximately 400 mL in volume, constructed of 20-micron mesh fabric (which is smaller than the diameter of most roots but large enough to allow the compounds of interest to flow unimpeded) and contained the same soil mix as the bulk soil. A monitoring well was placed in each bag which was similarly composed of an air stone attached to flexible, plastic tubing as described above.

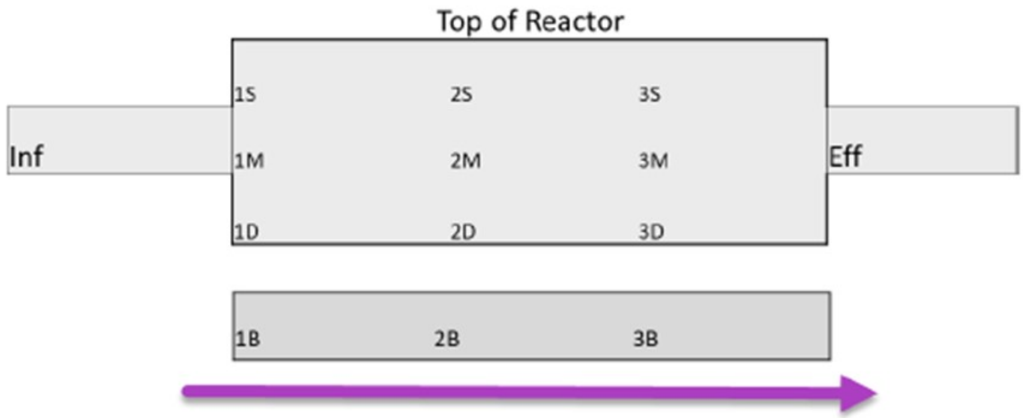

Figure 2. Sampling Setup of Test Reactors. Numbers represent horizontal position relative to influent, while letters represent depth from substrate surface. # 1 indicates samples 12.5 cm away from influent, # 2 indicates samples 25 cm from influent, and # 3 indicates samples 37.5 cm from influent. S indicates samples 8cm soil surface, M indicates samples 16 cm from the soil surface, and D indicates samples 24 cm from soil height, while B indicates a root-excluded sample (each at random height). Inf indicates the influent concentration and Eff indicates effluent concentration. The purple arrow indicates direction of flow through the reactor.

2.3. Experimental Methods

Porewater chemical concentrations and dissolved gas concentrations within the reactor and at the influent and effluent were measured using Ion Chromatography (Thermoscientific Ion Chromatography System) and Gas Chromatography methods (Shimadzu Gas Chromatography GC-2014 with AOC-5000 Plus Autosampler), with dissolved gas concentrations of nitrate, nitrite, ammonia, nitrous oxide, oxygen, carbon dioxide and methane measured to quantify transformational changes in biogeochemistry(see Appendix for further methodology). Flow into both reactors was stopped immediately before measurements were taken to examine the environment as a snapshot of a single, representative time point. Chemical concentrations were measured in all monitoring wells as well as in the influent and effluent by collecting a water sample with a glass syringe, ensuring no bubbles were present, and discarding a void volume to ensure only dissolved gas from the bulk soil was being collected. Samples for gas chromatographic analyses were placed into a glass GC vial that was immediately capped and checked to ensure bubbles were not present. Additional samples were also placed into a 2 mL centrifuge tube after being filtered with a 0.45-micron filter for Ion Chromatography measurements. Samples were stored underwater and refrigerated for up to 24 hours for GC measurement and up to two weeks for IC measurement. Sampling occurred on one sampling date in April 2017, 45days after creation of the mesocosms in March 2017, from all sampling ports in each reactor, as well as one sample from the influent and one sample from the effluent of each reactor for a total of 30 samples (15 per reactor).

2.4. Sample Analysis

Concentrations of nitrate, nitrite, and ammonium were measured on the Ion Chromatography instrument (Thermoscientific Ion Chromatography System). Gas concentrations, nitrous oxide, oxygen, carbon dioxide and methane were measured on the Gas Chromatograph (Shimadzu Gas Chromatography GC-2014 with AOC-5000 Plus Autosampler) after filling the headspace with pure N2 gas and allowing for equilibration. (see Appendix for further methodology)

Statistical analyses including Student t-tests, one-way ANOVA, linear regressions, multivariate correlations, and Mann-Whitney tests were conducted on normalized concentration values. Data were normalized relative to the influent concentrations, to compare values between the low flow reactor (RL) and the high flow reactor (RH) and to evaluate statistical differences between RL and RH, as well as among samples collected from the front, middle, and end of each reactor.

Comparisons were made using JMP Pro 13 software and R software. Given the small sample size, a p-value of 0.1 was used as a threshold for statistically significant data.

Results

3.1. Relationships Among Chemical Concentrations at Different Flow Rates

All influent concentrations were the same between RL and RH except for carbon dioxide (Table 1). The nine porewater samples taken from the bulk soil in each reactor were compared to determine if there were overall differences in mean chemical concentrations between each reactor at different flow rates. There were no significant differences between concentrations of methane, carbon dioxide, and nitrous oxide or oxygen concentrations between reactors (Table 1). Dissolved oxygen (DO) concentrations of bulk soil porewater samples met the standard of anaerobic conditions with an average concentration of 0.83mg/L in RH and 0.64mg/L RL. pH was also the same between both reactors at pH=7.6 (EPA, 2016).

A comparison of the concentration of nitrate between the influents and effluent of both reactors revealed that they were surprisingly consistent, with 48% nitrate removal in RL and 47% nitrate removal in RH from influent to effluent.

Table 1. Concentrations of chemicals in influent, effluent, and reactor mean of Low Flow and High Flow Reactor (mg/L). N.d. indicates locations where there is no data, n=1 for influent and effluent, n=9 for reactor.

Low Flow Reactor Concentrations (mg/L)			
Chemical Name	**Influent**	**Effluent**	**Reactor Mean**
Carbon Dioxide	0.25	n.d.	3.87±1.93
Oxygen	1.29	n.d.	0.62±0.11
Methane	0.0005	n.d.	0.19±0.16
Nitrous Oxide	0.00003	n.d.	0.00006±0.00002
Nitrate	0.13	0.07	0.012±0.010

High Flow Reactor Concentrations (mg/L)			
Chemical Name	**Influent**	**Effluent**	**Reactor Mean**
Carbon Dioxide	0.13	0.81	2.01±1.42
Oxygen	1.29	1.04	0.83±0.20
Methane	0.0005	0.02	0.21±0.34
Nitrous Oxide	0.00003	0.00004	0.00006±0.00003
Nitrate	0.13	0.07	0.059±0.036

3.2. Relationship between chemical concentrations and distance from influent.

There were however, significant differences in the subsurface chemical signatures, first, in the mean nitrate concentrations between RL and RH, with higher nitrate values ($p=0.032$, $n=9$) in subsurface porewaters of RH than in RL (Table 1).

Dissolved oxygen concentrations in porewater decreased over the length of the RL ($R^2=0.51$, $p=0.02$) (Figure 3). In contrast, oxygen concentrations did not change over length of RH ($R^2=0.004$, $p=0.85$) with n=3 at each data point within the reactor, n=1 at influent and effluent for RL (there was no effluent data for RH). Concentrations for methane, carbon dioxide, nitrate, and nitrous oxide were also analyzed across the length of the reactor but were not found to be correlated with length in either reactor.

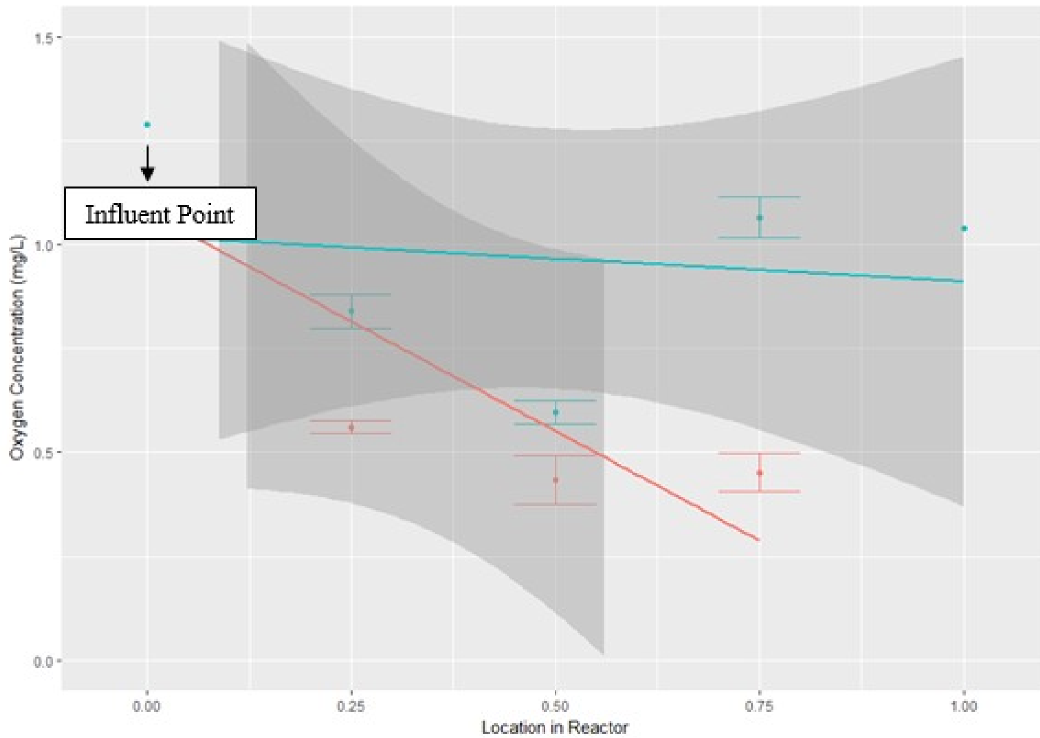

Figure 3. Oxygen concentrations as a function of location in High Flow Reactor (RH, blue) and Low Flow Reactor (RL, red). Error bars represent one standard deviation from mean.

3.3 Linear Relationships between Chemical Concentrations

In order to examine the relationships between chemical concentrations (after they were normalized relative to influent concentration), and how those relationships might differ at different flow rates, concentrations of each nutrient or greenhouse gas were compared to the concentrations of the other nutrients or greenhouse gases at the same location over the length of each reactor.

In RL, all oxygen concentrations were found to be anaerobic. These oxygen concentrations were also significantly negatively correlated with carbon dioxide (R^2=0.394, p=0.05) and nitrous oxide (R^2=0.315, p=0.09), but significantly positively correlated with nitrate R^2=0.633, p=0.006;Figure 4). Additionally, carbon dioxide was found to be significantly positively correlated with methane (R^2=0.571, p=0.012), and significantly negatively correlated with nitrate (R^2=0.276, p=0.0006), and nitrous oxide (R^2=0.367, p=0.0632; (Figure 4). No relationships were observed between oxygen and methane, methane and nitrate, methane and nitrous oxide, or nitrous oxide and nitrate concentrations (Figure 4).

In the high flow rate reactor (RH), fewer statistically significant relationships existed among chemical species than in the Low Flow Reactor (RL). Oxygen was again found to be negatively correlated with carbon dioxide (R^2=0.56, p=0.007) and positively correlated with nitrate (R^2=0.58, p=0.006;Figure 4). Carbon dioxide was found to be negatively correlated with nitrate (R^2=0.81, p=0.0002;Figure 4). No relationships were observed between oxygen and methane, carbon dioxide, nitrate, or nitrous oxide or for carbon dioxide and nitrate or nitrous oxide as well as nitrate and nitrous oxide (Figure 4).

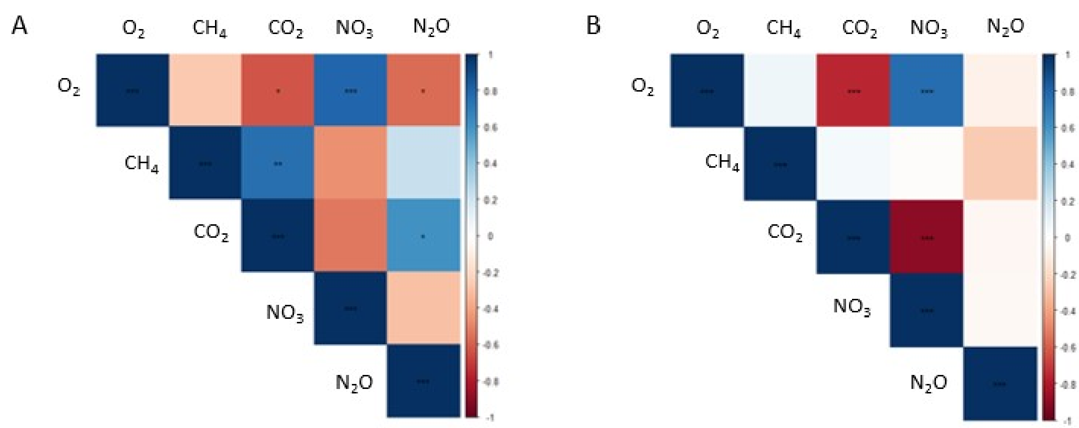

Figure 4. Multivariate correlation plots of Oxygen, Nitrate, Methane, Carbon Dioxide, and Nitrous Oxide Concentrations for RL (A) and RH (B). n=9 for both reactors. Dark blue indicates an r-value close to +1 indicating a highly positive correlation, while white indicates r-values around 0 indicating no relationship, and dark red indicate values around -1 indicating a highly negative correlation. Stars indicate p-value with one star indicating a value less than 0.1, two stars indicating a value less than 0.05, and three stars indicating a value less than 0.01.

3.4: Relationship between the presence of Roots and GHG Cycling

Regardless of flow rates, the nitrous oxide concentrations in the subsurface soil which contained roots were lower than concentrations in the rootless soil bags (p

=0.04 RL, 9 root-exposed samples and 2 root-excluded samples and p=0.09 RH, 9 root-exposed and 3 root-excluded samples; Figure 5). Methane concentrations in the subsurface were higher when roots were present as compared with concentrations in soil where roots were not present (p=0.07, 9 root-exposed samples, 2 root-excluded samples and p=0.09, 9 root-exposed and 3 root-excluded samples; Figure 5). There was no statistical difference between oxygen, carbon dioxide or nitrate concentrations for either reactor regardless of the presence or absence of roots (Figure 5).

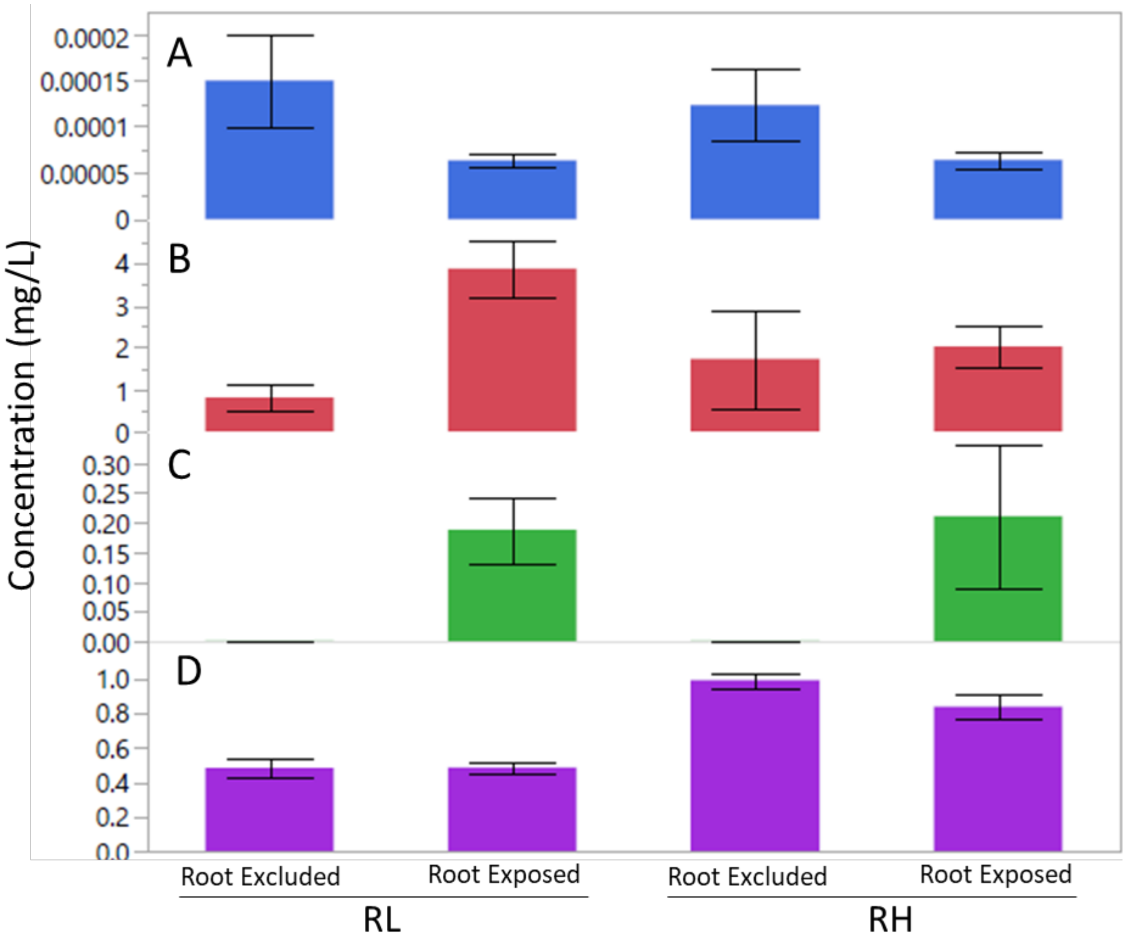

Figure 5. Mean Dissolved Gas Concentrations in porewater exposed to roots and excluded from roots for Nitrous Oxide (A), Carbon Dioxide (B), Methane (C), and Oxygen (D). Root exclusion was performed in-situ using sampling ports within 20-micron mesh bags to allow chemical flow through but prevent growth of roots (n=2 for RL and n=3 for RH). Samples from porewater exposed to roots were taken from the bulk soil (n=9 for each reactor). Error bars indicate one standard deviation from mean.

Discussion

The growing quantity and declining quality of stormwater runoff due to climate change and nutrient loading from increasing agricultural production worldwide will have a marked impact on the effectiveness of constructed wetlands and their potential contributions to greenhouse gas emissions. The many environmental parameters controlling biogeochemistry in constructed wetlands, specifically aerenchymal roots and flow rate, may serve as tools for mitigating these emissions and effluent nutrient concentrations, however the effects of these two parameters have been difficult to clarify (Hiraishi et al. 2013; Maucieri et al. 2017). Our study indicated that groundwater flow rate and presence of roots interact to control the biogeochemical cycling of nitrate in constructed wetland through their independent impacts on subsurface concentrations of gases.

Since the presence or absence of dissolved oxygen serves as a driving force for numerous biochemical reactions, it is important to understand the dynamics of its concentration throughout a constructed wetland. Over the flow path of the Low Flow Reactor, dissolved oxygen concentrations were variable, but overall decreased between inflow and outflow ports. However, in the high flow reactor, the oxygen concentrations remained stable and did not decrease. This pattern difference is likely due to the hydraulic retention time (HRT) of each reactor. The Low Flow reactor had an HRT twice as long as the faster High Flow Reactor. Since there is no turbulence in our reactors, there is not an identifiable source of reaeration in these systems.

In streams, it has been well documented that increasing velocities are correlated with greater oxygen content, largely due to increased turbulence and mixing of the surface water with the atmosphere at the air-water interface which then reaerates the water (Thyssen and Erlensen, 1987; USGS 2011). This mechanism is unlikely to be relevant in the reactors as the there is no turbulence, and flow rates are much slower. Instead, removal of oxygen by microbial metabolism as the water flows past the soil particles is probably driving the oxygen depletion in RL. The shorter the retention time and faster the flow, as in RH, then the less time there is for microbes to interact with the water and use up the oxygen. Understanding this process may be useful in the future, as flow rates in constructed wetlands can be controlled and slowing flow rates can encourage anoxia.

Our study also confirmed that increasing flow rate is associated with differences in biogeochemical nitrogen transformations, most notably, higher nitrate concentrations, even though concentrations of denitrification products, like nitrous oxide, remained statistically similar. This may be explained by the decreased denitrification efficiency of a water system when exposed to higher concentrations of nitrate, as concluded by Hall and collaborators in their study of natural stream systems (Hall et al. 2009). Even though nitrate concentrations for both systems were initially the same, more nitrate remained within the reactor at higher flow than at lower flow. Howarth et. al (2006) also determined that increasing water flow rates increased nitrate concentrations overall, indicating detrimental effects for nutrient removal with increasing flooding events, and this finding was also confirmed in our controlled wetland mesocosms (Howarth et al., 2006).

Since our study did not find differences in concentrations of nitrous oxide, it is possible that complete denitrification from nitrate to nitrogen gas may have occurred. However, since we also did not determine concentrations of nitric oxide, it also remains a possibility that this was the gas formed in our mesocosms. This has important implications for GHG emissions in constructed wetlands, which may be forming even if they are not present in the effluent of the wetlands. Concentrations of nitrate within reactors, as shown in this study, may be used as an indicator of this GHG formation.

Given the observed relationships between oxygen and nitrate, as well as oxygen and nitrous oxide, it is likely that we have also observed a threshold for oxygen control of denitrification within our system. Rivet et. al in 2008 determined that concentrations of less than 1mg/L of oxygen were low enough for denitrification (Rivett et al. 2008). In this study, we consistently observed concentrations below 1 mg/L. The mean concentration of oxygen in RL was at 0.62 mg/L while the mean concentration in RH was 0.83mg/L (Table 1), indicating both reactor systems can support denitrification. Although the mean concentrations were within one standard deviation of one another, the presence of microsites within each reactor with differing concentrations may be the cause of this change in nitrate concentration. A study by Nakajima et. al (1982) concluded that although 0.19 mg/L to 2.01mg/L O_2 concentrations could support denitrification, the rate of denitrification is halved at 0.63mg/L O_2 (Nakajima et. al, 1982). Given that mean concentration of oxygen in RH was higher than this number, this may account for the lack of relationship between oxygen and nitrous oxide in RH, while whereas a significant relationship was observed in RL.

In addition to the relationship between oxygen and nitrate, we also observed two significant correlations in RL that did not exist in RH, specifically a negative relationship between oxygen and nitrous oxide as well as a positive relationship between carbon dioxide and methane. The dependence of nitrous oxide on dissolved oxygen was also found by Rosamond et. al (2012) in riverine environments who determined that this was likely due to the formation of nitrous oxide in the hypoxic and anoxic areas. This may explain the lack of a relationship in our higher flow rate reactor as there are higher oxygen concentrations in this reactor and thus fewer hotspots for denitrification reactions to occur.

The relationship between carbon dioxide and methane was also observed only in the lower flow rate reactor (LR). Several studies have confirmed that different environmental parameters are involved in controlling carbon dioxide and methane in wetlands including dissolved oxygen and bacterial abundance for carbon dioxide and oxidative-reductive potential for methane (Wu et al. 2007), often with opposing relationships between carbon dioxide and methane fluxes (Batson et al. 2015). However, studies also indicate that anaerobic systems produce both carbon dioxide and methane whereas aerobic systems only produce carbon dioxide (Batson et al. 2015). Given the placement of our reactors on opposite sides of the oxidation threshold, it is likely that the relationship seen in the Low Flow Reactor is also a side effect of lower oxygen levels, whereas the lack of relationship in the High Flow Reactor could be contributed to the occurrence of both aerobic and anaerobic bacterial reduction.

In addition to the many relationships between oxygen and nitrate, there was a strong significant relationship between carbon dioxide and nitrate only in the High Flow Reactor, while a negative, yet insignificant correlation was documented in the Low Flow Reactor. One possible explanation for this relationship could be the production of malate associated with higher carbon dioxide concentrations in plants, which is associated with a lack of production of nitrate (Purvis et. al, 1974). Malate is a product of plant metabolism; however, it is less common in anoxic soils due to its presence in the C4 pathway, which is not common in waterlogged, and thus anoxic, soils (Purvis et. al, 1974). Given the higher dissolved concentration of carbon dioxide in RL than in RH (Figure 4), and the lower mean concentration of nitrate in RL than in RH, malate production may be higher in RL than in RH. However, the lack of significant correlation between carbon dioxide and nitrate concentrations in RL indicates that there may be more factors impacting these concentrations (Figure 3).

One other factor impacting the relationship between carbon dioxide and nitrate concentrations in RH is the relationship between carbon dioxide and oxygen concentrations. This relationship is stronger in the High Flow Reactor, which could lead to the correlation between carbon dioxide and nitrate in the High Flow Reactor. Future studies should be conducted to determine if flow rate may lead to a differential malate, and thus nitrate plant production.

The presence of *S. acutus* aerenchymal roots in the soil was associated with an increase in methane and decreased nitrous oxide and other dissolved gas concentrations in the soil solution. While an increase in nitrous oxide emissions is found in some studies (Jorgenson et al. 2012), due to the physical transport of gases typical of aerenchymal roots, previous studies have also shown the same result as we did (Wang et al. 2008, Silvan et al. 2005). Wang et. al, 2008 found that nitrous oxide concentrations in *Phragmites australis* planted wetlands were up to 38.2 mg N_2O m^{-2} d^{-1} less when compared to unplanted wetlands of *P. australis* in their study of microcosm wetlands (Wang et al. 2008). Another study that came to a similar result was Silvan et. al, 2005, who found that *Eriophorum vaginatum* presence in their restored natural peatland had the effect of reducing nitrous oxide emissions as compared to non-planted peatlands and hypothesized that plants serve as a competitor for inorganic N. Silvan et. al, 2005 also found that the presence of aerenchymal plants leads to a decrease in nitrous oxide emissions as plants compete for use of inorganic N with microorganisms by using N in biomass production. Their findings confirm that aerenchymal plants competitively uptake nitrate which thus cannot be used in microbial denitrification (Silvan et. al, 2005). Our findings, that higher concentrations of nitrous oxide are present in porewater when roots are not present in both flow rate reactors, seem to support this result. Interestingly, we found no significant differences between nitrate concentrations in porewater between root excluded and exposed locations, therefore it is likely that the difference driving nitrous oxide production is the use of nitrate by microorganisms and plants.

While the presence of *S. acutus* roots reduced nitrous oxide, they enhanced the concentrations of methane in our study. The difference in the relationships is likely due to the different controls on nitrous oxide and methane production. While nitrous oxide emissions appear to be controlled by the presence of organisms which use inorganic N, methane production appears to be associated with the processes of primary production. Our findings support the observations of Whiting and Chanton (1993). Their study describes methane emissions from several different natural and agricultural wetlands with aerenchymal plants and determined that those wetlands with higher net primary production are also the areas where methane emissions are the greatest. It may be that root exudates from the plants provide a ready source of carbon for methanogenic bacteria, fostering the methane production.

While the Whiting and Chanton (1993) study focused on methane emissions, another study conducted by Stanley and Ward (2010) looked at the relationship between dissolved gas concentrations of methane and the presence of different species of vascular plants. They found that while the relationship was not clear, different communities of vascular plants did, in fact, cause differences in these gas concentrations. Their findings suggest that in addition to methane emissions, dissolved gas pools are also differentially affected by the presence of different species of aerenchymal plants.

Although aerenchyma are well known for transporting oxygen from the surface to the subsurface (Takahashi et. al 2014, Evans et. al 2004), we found no relationship between oxygen concentrations and root presence in either of our systems. Both reactors had low oxygen concentrations. This may be due to our in-situ methods. Although roots were excluded by our bags, it is possible that there was some exchange of oxygen from roots through bags, based on evidence that the oxygen produced from *Schoenoplectus* can travel from roots to root-excluded locations (Bezbaruah and Zhang 2005). Similarly, this process may also explain the similarity in carbon dioxide concentrations for both locations. Future studies should be conducted to better determine the relationships between *S. acutus* presence and dissolved gas concentration travel as well as how this will impact overall emissions. The results of this study indicate that planted and unplanted areas in constructed wetlands are likely a good way to promote nitrate removal and denitrification, while also promoting the removal of methane. Further studies on carbon and nitrogen availability in wastewater and soil should be used to make determinations on whether to plant *Schoenoplectus actus*.

Conclusions

Agricultural Constructed Wetlands represent a low cost and environmentally friendly method of wastewater treatment. However, it is important to understand the environmental parameters driving the effectiveness of this treatment method. With climate change, we expect to see increases in nitrate loading and unpredictable rain events, and these shifts will likely impact the effectiveness of constructed wetlands to remove nitrate as well as their propensity to generate greenhouse gases. In particular, the impact of flow rate and presence of aerenchymal roots remain unresolved and will be crucial in a system disrupted by climate change. In this study, we designed and built two mesocosms modeling continuously-fed constructed wetlands to determine the impacts of these two parameters.

This study addressed two major research questions: 1) What is the relationship between flow rate and nutrient cycling in constructed wetlands? and 2) What is the relationship between the presence of aerenchymal roots and nutrient cycling in constructed wetlands. We determined that the higher flow rate increases both subsurface concentrations of oxygen and nitrate in porewater and overall less predictability of chemical concentrations. Since climate change is likely to bring higher flows in these areas, we are likely to lose some of the flow rate control on denitrification. However, since we can control plant species and placement in constructed wetlands, we may be able to use the results from this study, that aerenchymal *S. acutus* plants increase methane emissions and decrease nitrous oxide emissions, to control this denitrification. Future studies should be conducted to determine the potential of *Schoenoplectus actus* to control denitrification in constructed wetlands, as well as how the plant's density effects these controls.

APPENDIX

This appendix is a collection of work that was conducted as part of the over-arching research project. Some elements, such as the reactor set-up and calibration of the equipment, were critical to this research project whereas other elements represent preliminary investigations into other related research questions.

Preliminary Overview of MS Research to Date

OVERALL PROJECT GOAL

This master's research is part of a broader lab-wide examination by Dr. Matthew Reid on constructed wetlands work to process wastes and produce greenhouse gases. Specifically, this study focuses on the role of wetland plants in physical and chemical processes in constructed wetlands.

SPECIFIC OBJECTIVES

The specific objective of my master's project is to use 2 bioreactor wetland microcosms to figure out the differences in physical and chemical processing between constructed wetland systems with and without plant roots by comparing data from monitoring wells in root-exposed areas and root-excluded bags within the reactor.

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    - 3c: May Nitrate and Gas Chemistry
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- **PART VIII: PRELIMINARY RESULTS SUMMARY**
- **PART IX: STOMATAL CONDUCTANCE WORK (FALL 2017)**
    - 9a: Construction & Setup of New Reactor
    - 9b: Preliminary Bromide Tracer Test
    - 9c: In-Depth Bromide Tracer Test

TIMELINE OF SELECTED EVENTS TO DATE: Winter 2017-Fall 2017

Winter 2017

- <u>January</u>
    - Preliminary Bromide Tracer Tests
    - Building/Setup Reactors

Spring 2017

- <u>February</u>
    - IC Manual

- Soil Moisture Probe Placement & Testing
    - Planting Reactors
    - Training MEng Student on Bromide Tracer Tests
- March
    - Influent and Effluent Tests
    - Nitrate Test
    - 1st Refrigerator Addition
    - 2-3x monthly Water + Wastewater Replacement
    - Flux Chamber Set 1 Preparation
- April
    - Influent and Effluent Tests
    - Nitrate + Gases Test
    - 2-3x monthly Water + Wastewater Replacement
- May
    - Influent and Effluent Tests
    - Nitrate + Gases Test
    - 2-3x monthly Water + Wastewater Replacement

Summer 2017

- June
    - Intensive Influent and Effluent Testing
    - 2nd Refrigerator Addition and Maintenance
    - Push Pull Test 1
    - Push Pull Test 2
    - GC Calibrations
    - Weekly Water + Wastewater Replacement
    - Flux Chamber Set 2 Preparation
- July
    - Influent and Effluent Testing
    - Push Pull Tests 3 & 4
    - Push Pull Tests 5 & 6
    - Proposal for Fall 2017 Work
    - GC Calibrations
    - O2 in Reactor Tests
    - Root-Excluded Bag Tests
    - Injection bag Sampling
    - Summer Nitrate + Gases Test
    - Weekly Water +Wastewater Replacement

Fall 2017

- August
    - Building/Setup New Reactor

- Preliminary Bromide Tracer Test
- In-Depth Bromide Tracer Test
- Flux Chamber Set 3 Preparation

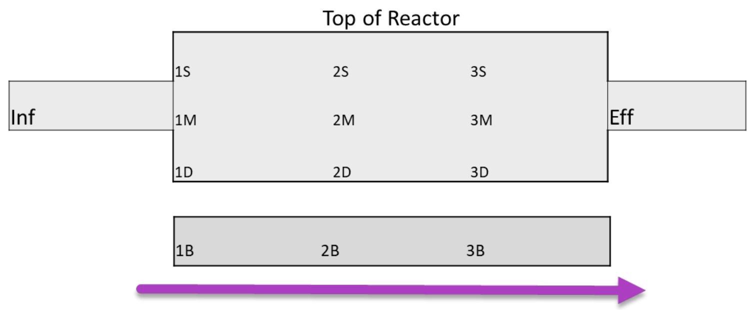
PART I: ESTABLISHMENT OF 2 CONTINUOUS FLOW BIOREACTOR WETLAND MICROCOSMS

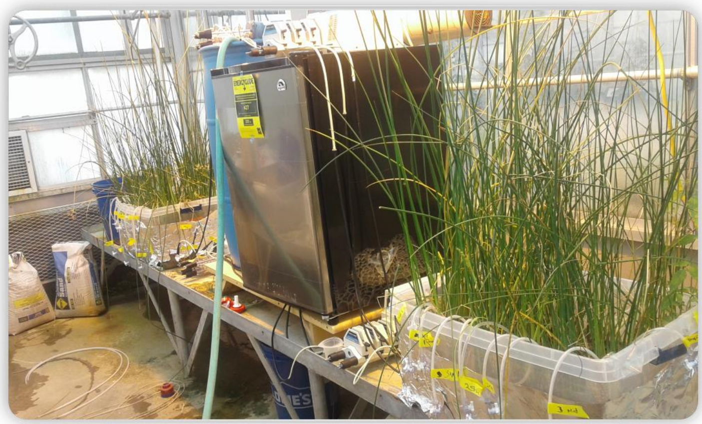
1a. Construction/Planting Reactors (Winter/Spring 2017 o *Time:* 100 hours)

- Goal: To create 2 mesocosm wetland reactors in greenhouses for experiments.
- Method: 2 Reactors were built by drilling holes into the sides of the reactors, with 3 influent ports and 1 effluent port.
    - 3 root excluding bags containing a carbon amendment and sand were set up in each reactor. Sampling ports were added in the following configuration with 3 root-excluded bag sampling ports that were 500 mL in volume, with a 0.3 porosity.
    - 12 application wells were set up for inserting gas in water. All 12 wells were 1/8in ID tubing connected to an air stone. 3 of the wells were contained within a 20-micron fabric bag with soil, these were setup as seen in the figure

below.

Sampling Setup:





- Piping and refrigerated pumps set up to allow 2 different flow regimes (5m l in reactor 1, 20 ml/min in reactor 2
- Reactors were seeded with 10% by volume inoculum soil, 5% by volume ground leaf litter, and multipurpose Lowes sand. *Scirpus actus* were planted, with 24 in each reactor. Plants grew for 1 month before routine monitoring occurred.

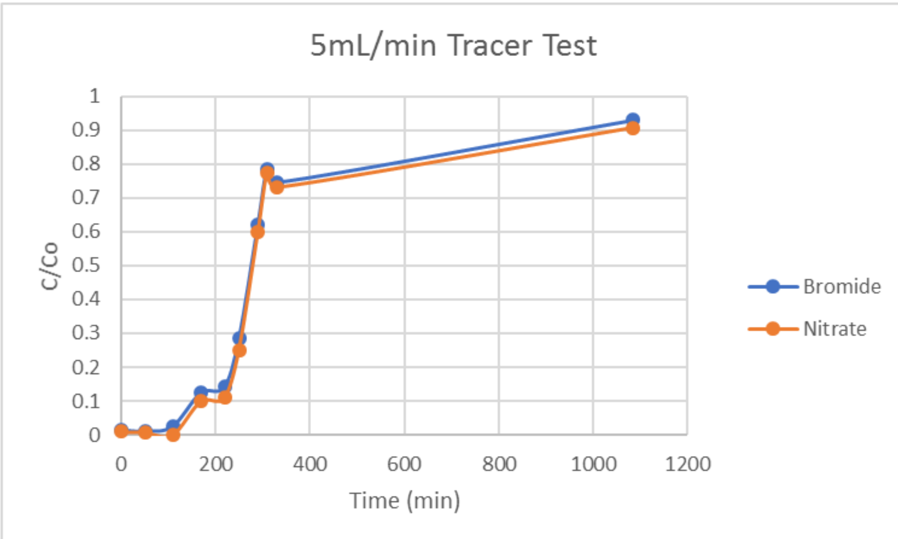
- **Refrigerator Setup, Change in Influent Regime**
  - <u>Goal:</u> Set up the refrigerator with holes and change the influent to 16 tubing with 1.3mL/min per line.

- Methods: Researched refrigerator to add to system that would not have coolant in the door (1 unsuccessful refrigerator), researched and drilled holes in doors and installed refrigerator in current position, added influent wastewater and made sure the influent rate was the same as expected while changing out influent tubing.

- **1B. SOIL MOISTURE PROBE TESTING**
  - Goal: To monitor soil moisture in intermittent flow pattern reactor
  - Method: Soil moisture probes were also added powered by Arduino software, and an intermittent flow pattern in reactor 2 was obtained using pro-coda software.
  - Result: Corrosion occurred with soil moisture probes, causing them to work incorrectly, and intermittent flow pattern was not used. **(see photo)**

- **1c. Bromide Tracer Tests to Evaluate Flow Regimes** (Winter 2017 o *Time:* 40+ hours**)**
  - Goal: preliminarily characterize the flow pattern of the reactor before planting and adding carbon amendment.
  - Hypothesis: System will behave as a PFR.

- **Method:** Tests were run at 5mL/min and 20 mL/min using a 5mM Bromide and 5mM Nitrate solution with samples taken intermittently. 5mL/min experiment was run for 20 hours and the 20mL/min experiment as run for 5 hours.
- **Results:** The reactor appears to flow as a PFR. Initial tests (see figures) showed good flow but indicated more soil needed in reactors.



- **1d. Root-Excluded Bag Tests** (Summer 2017 o *Time:* 8 hours**)**
  - Purpose: To determine if the water that passes through the bulk soil also passes through the root-excluded bags, and if the water chemistry remains the same.
  - Methods: 1 100-micron bag, 1 20-micron bag, and 1 bulk soil sampling port were placed in a reactor with a 6-hour retention time. A 5mM

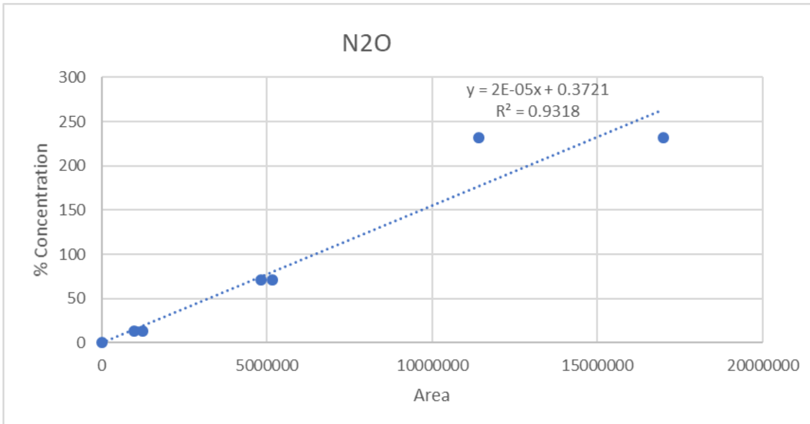
bromide was flown through at 5mL per minute for 4 days, with samples taken once a day from each port. Samples were analyzed on the IC for bromide concentration.
  - Hypothesis: Root-excluded bags will have the same concentration of bromide as the bulk soil.
  - Results: After 1 day, the concentrations in the bulk soil, 100-micron bag, and 20-micron bag were all equal to the concentration of the influent 5mM bromide solution. The concentrations remained constant for the rest of the 4-day period.
- **1e. Oxygen Concentration in Reactor Tests** (Summer 2017 o *Time:* 6 hours**)**
  - Purpose: The purpose of this test was to determine if the porewater was a truly anoxic environment after the placement of nitrogen bubbled influent water.
  - Hypothesis: Oxygen will be present at low concentrations.
  - Methods: Pore water samples were taken from 3 different locations in the reactor with a glass syringe and analyzed on the GC for O2 content.
  - Results: Oxygen was present in all 3 locations, but at below 1mg/L concentrations.

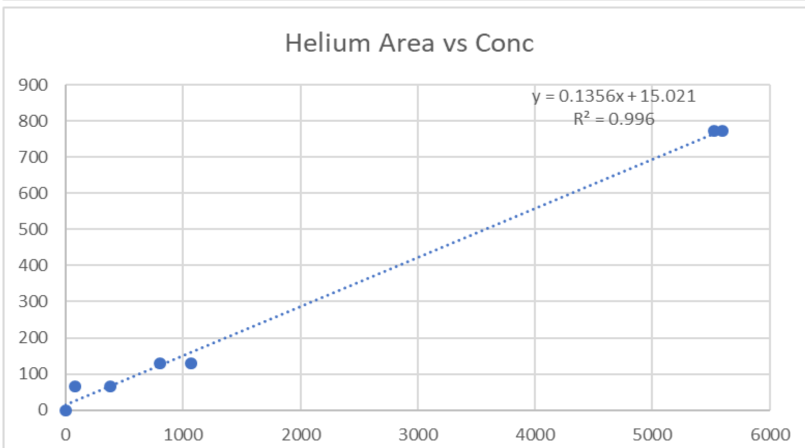
Part II: Instrumentation calibration/ set-up

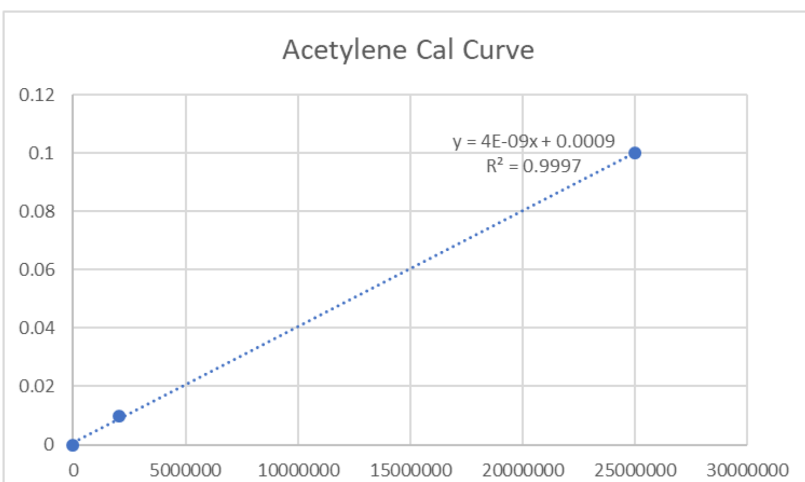
- **2a. Thermoscientific IC Method Development, Calibration, and Maintenance** (Summer 2017 o *Time:* 40 hours**)**
  - Purpose: Develop a method for use of the Ion Chromatography machine to reliably analyze samples
  - Method & Results: See Appendix II for most recent IC method guide.

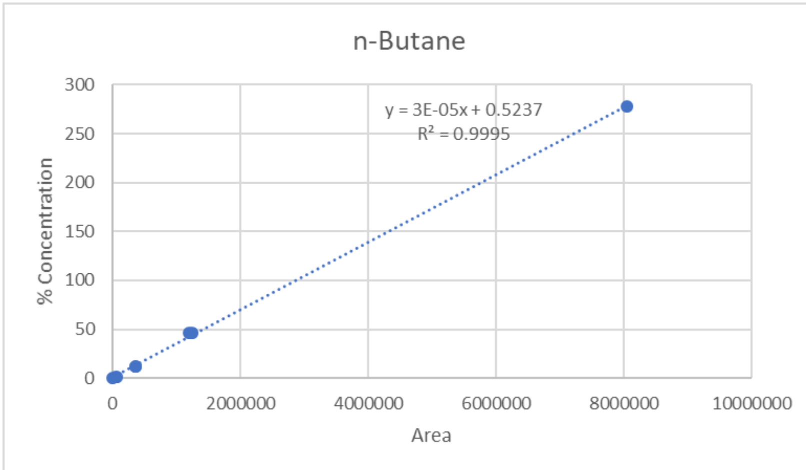
- **2b: Shimadzu GC Calibration Curve Development** (Summer 2017 o *Time:* 40 hours**)**
  - Purpose: Development of calibration curves for ethylene, ethane, propane, n-Butane, nitrous oxide, helium, and acetylene for analysis of GC data.
  - Methods: At least 3 different, known concentrations were analyzed on the GC for ethylene, ethane, propane, n-Butane, nitrous oxide, helium, and acetylene, with each concentration analyzed twice to ensure accuracy. Known concentrations were plotted with areas for those concentrations to develop calibration curves for each.

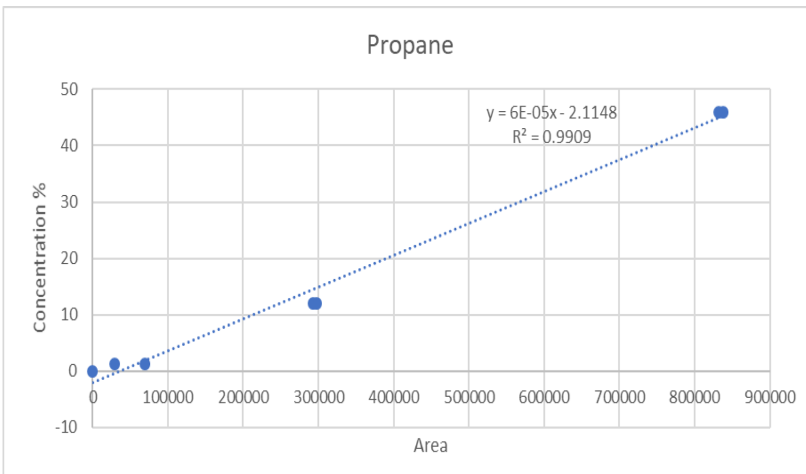
○ <u>Results:</u> Graphs and calibration curves for ethylene, ethane, propane, n-Butane, nitrous oxide, helium, and acetylene are depicted below.

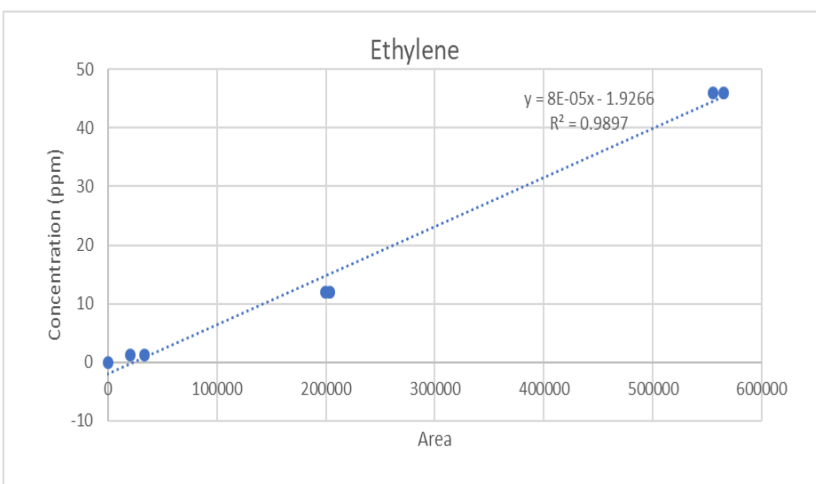


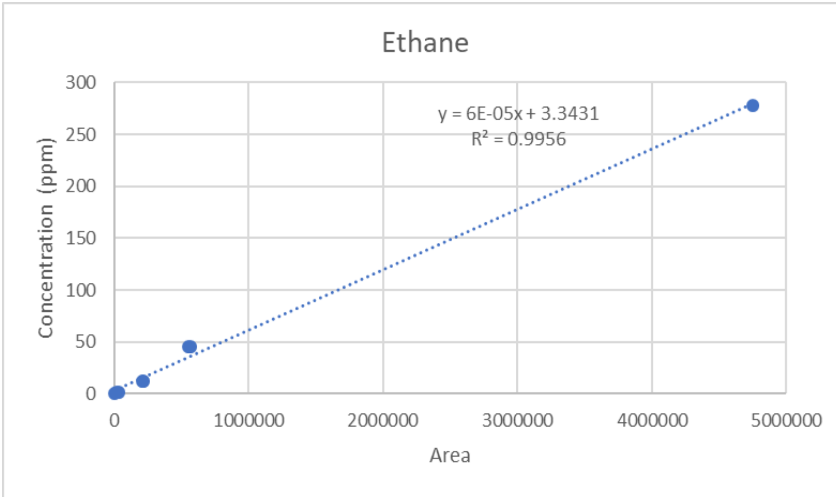





n-Butane
y = 3E-05x + 0.5237
R² = 0.9995
% Concentration
300
250
200
150
100
50
0
0
2000000
4000000
6000000
8000000
10000000
Area


Propane
y = 6E-05x - 2.1148
R² = 0.9909
Concentration %
50
40
30
20
10
0
-10
0
100000
200000
300000
400000
500000
600000
700000
800000
900000
Area


Ethylene
y = 8E-05x - 1.9266
R² = 0.9897
Concentration (ppm)
50
40
30
20
10
0
-10
0
100000
200000
300000
400000
500000
600000
Area



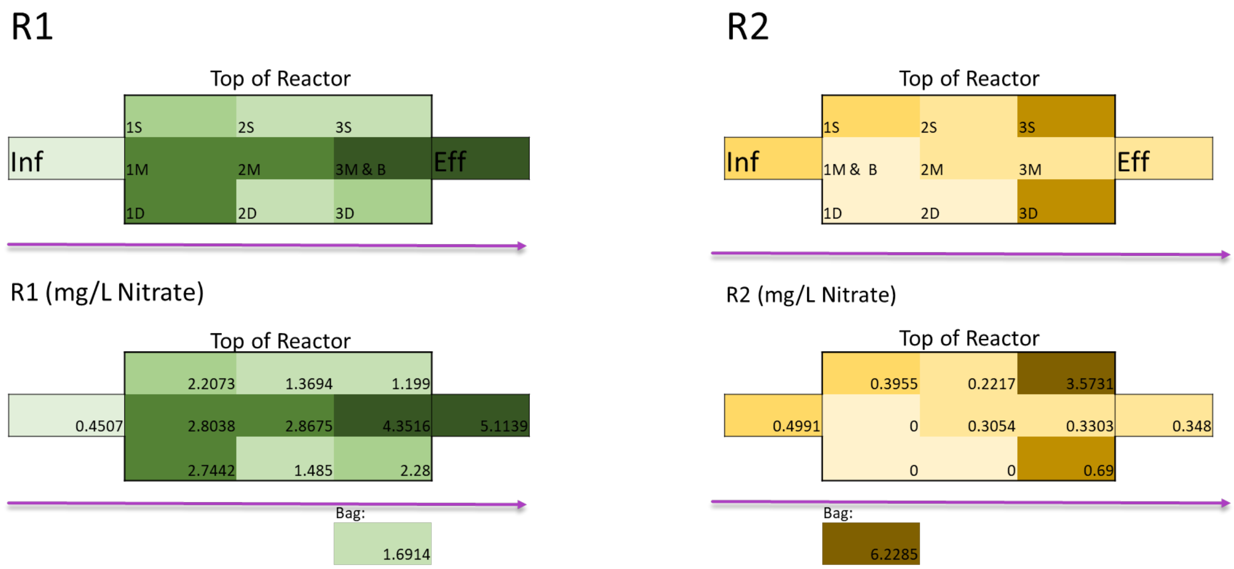
- **2c. Shimadzu TOC/TN**
  - Purpose: Measuring Total Carbon, Total Organic Carbon, and Nitrogen in Influent, Effluent, and within the reactor
  - Methods: Standards made for carbon, total organic carbon, total for each relevant experiment, particularly inflow outflow chemical comparisons and modification

PART III. RESEARCH EXPERIMENT #1: COMPARING WATER AND GAS CHEMISTRY BETWEEN WETLAND SOIL AND ROOT-EXCLUDED BAGS

****In All tests B1, B2 and B3 are root-excluded bags. All others are root-exposed wells****

- **3a. March Nitrate-Only Monitoring Well Test** (Spring 2017 o *Time:* 15 hours)
  - Purpose: Determine the amount of nitrate present in different sampling ports within reactors 1 and 2.
  - Hypothesis: Nitrate concentrations would be different in R1 and R1 due to different flow regimes, there would also be differences throughout each reactor.
  - Method: Samples were taken at all sampling points for both reactor 1 and 2 and analyzed using the IC for nitrate concentration.

- Key Results:
  - Root Excluded bag in R1 was nitrate poor while Root-Excluded bag in R2 was nitrate rich, this may be due to a higher flow rate in R2.



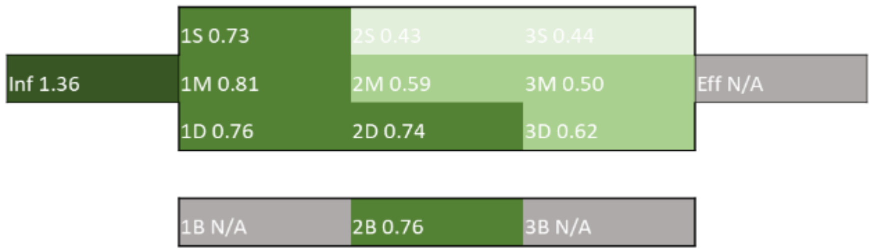
Darker colors indicate higher concentrations of nitrate

- **3b. April Nitrate + Gases Monitoring Well Test** (Spring 2017 o *Time:* 40 hours)
  - Purpose: Expand experiment 3A to more parameters including oxygen, nitrate, methane, ammonia CO2, N20, and Total Carbon for two different flow regimes.
  - Hypothesis: Roots remove gases therefore higher concentrations will be measured in non-root bags
  - Method: Samples were taken from all sampling ports in Reactor 1 and Reactor 2 as well as Root-Excluded ports in each reactor. They were analyzed for nitrate and nitrite on the IC, using the GC to analyze methane, CO2, O2, and N2O concentrations, and using the TOC/TN to determine total carbon concentrations.
  - Key Results: (where N/A=Concentration Too Low for Detection)
    - O2 levels are opposite in R1 and R2
    - CO2 levels are generally lower in root-excluded bag monitoring wells
    - No Methane in Bags

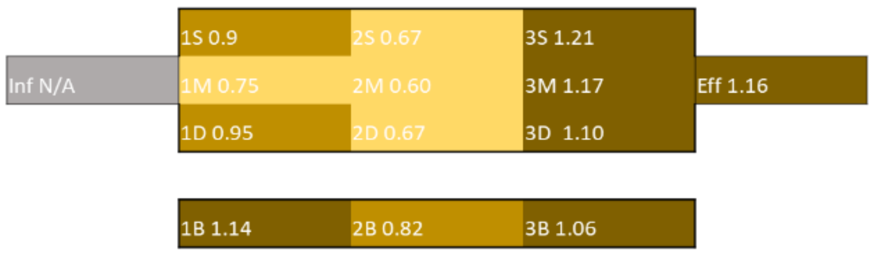
- Nitrate-N highest in bags
- Ammonia was not present in any monitoring wells

O2 Data in mg/L

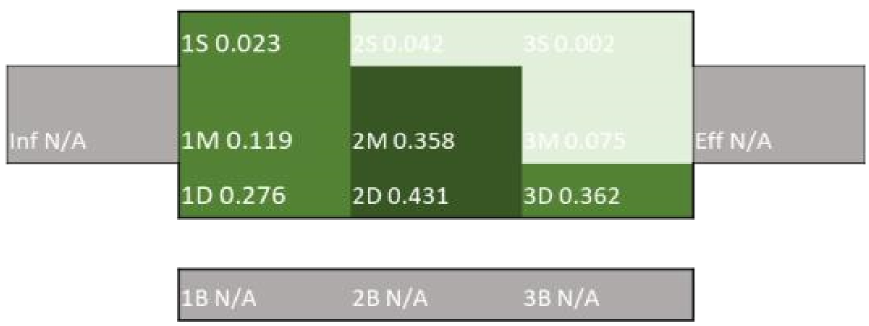
R1



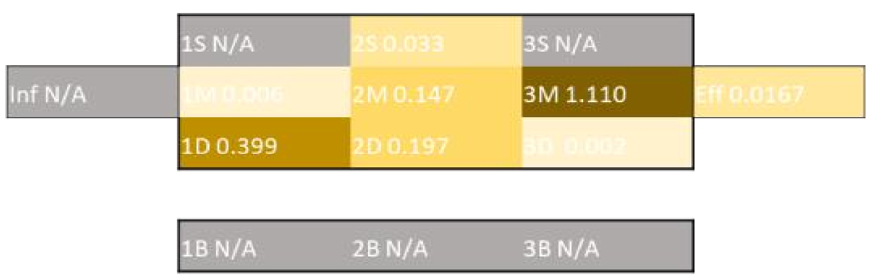
R2



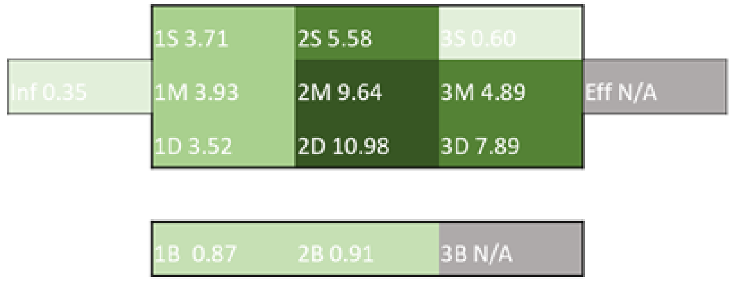
Methane Data in mg/L



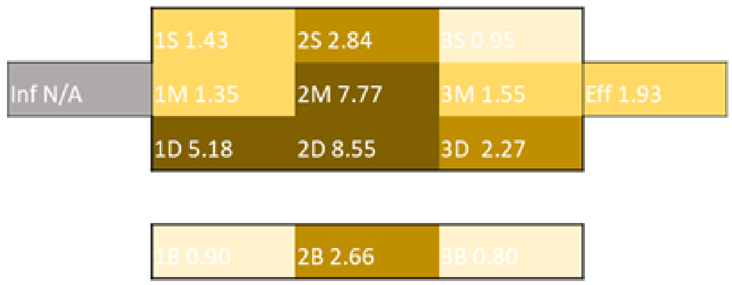
R2



CO2 Data (mg/L)

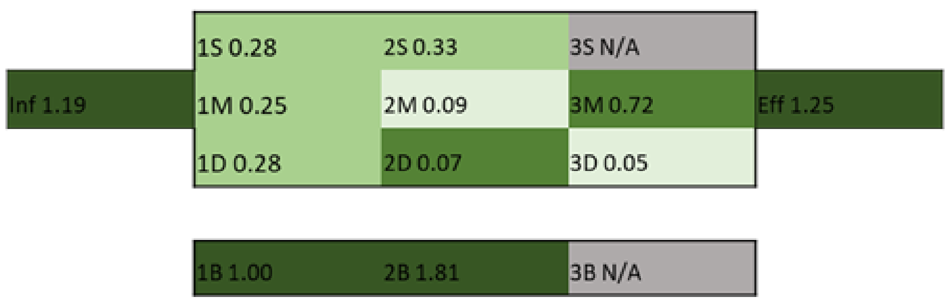


R2

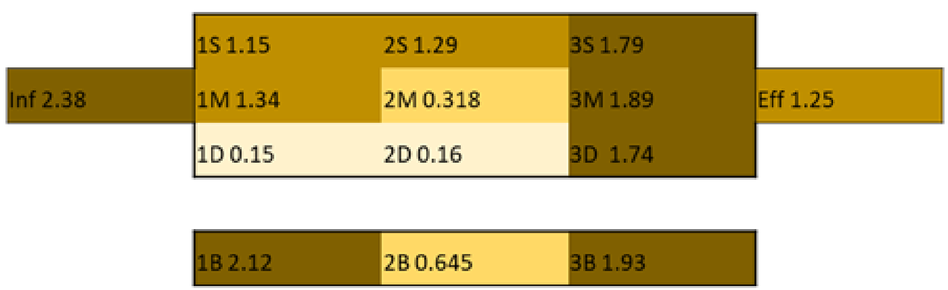


Nitrate-N in (mg/L)

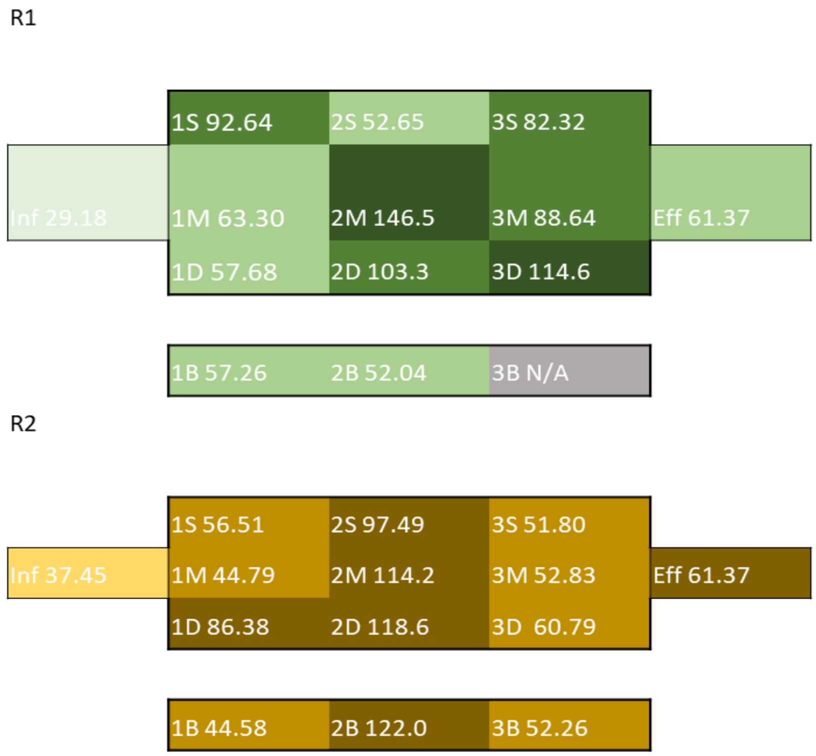
R1



R2

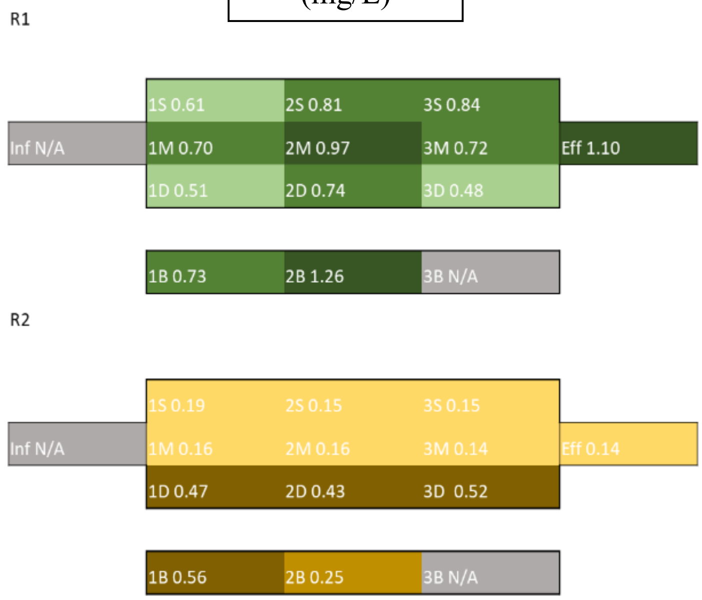


Total Carbon Data (mg/L)

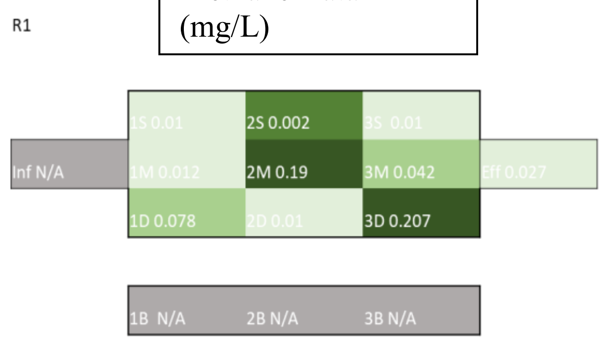


- **3c. May Gases Monitoring Well Test** (Spring 2017 o *Time:* 40 hours**)**
  - <u>Purpose:</u> Replicate Experiment 3b to see if same findings occurred on determine the concentrations of oxygen, methane, CO_2, O_2, and N_2O of the sampling ports for Reactor 1 and Reactor 2.
  - <u>Hypothesis:</u> Roots remove gases therefore higher concentrations will be measured in non-root bags
  - <u>Method:</u> Samples were taken from all sampling ports in Reactor 1 and Reactor 2 as well as Root-Excluded ports in each reactor. They were analyzed using the GC for methane, CO_2, O_2, and N_2O concentrations.
  - <u>Key Results:</u>
    - CO_2 concentrations were lowest in root-excluded bags
    - Methane concentrations were lowest in root-excluded bags
    - N_2O was highest near the influent of the reactors (still need to continue analyzing this data)

O2 Data
(mg/L)

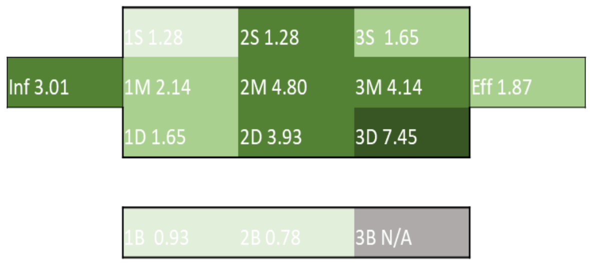


Methane Data
(mg/L)



CO2 Data (m/L)

R1



- **3d. Summer Nitrate/Nitrite/Ammonia + Gases Test** (Spring 2017 o *Time:* 10 hours**)**
  - Purpose: The purpose of this experiment was to determine the concentrations of oxygen, nitrate, methane, CO2, O2, N2O, nitrate, nitrite, and ammonia in each of the sampling ports for Reactor 1 (the reactor used for all push-pull tests)
  - Hypothesis: CO2, Methane, and Nitrate levels will be like those found in previous tests.
  - Method: Samples were taken from all sampling ports in Reactor 1 and Reactor 2 as well as Root-Excluded ports in each reactor. They were analyzed for nitrate and nitrite on the IC, using the GC to analyze methane, CO2, O2, and N2O concentrations
  - Key Results:
    - Methane concentrations were lowest in Root-Excluded Bags
    - CO2 concentrations were lowest in Root-Excluded Bags
    - Nitrate levels were highest in Root-Excluded Bags
    - Ammonia was not present in any monitoring wells.

	Summer Routine Monitoring Results					
	mMol/L methane	mMol/L CO2	mMol/L N2O	Nitrate-N mMol/L	Nitrite-N mMol/L	Ammonia-N mMol/L
M2	0.105953359	1.232882483	0.002607672	0.602903226	0.703043478	b.d.l
D3	0.014415788	0.782420661	0.070864979	10.90193548	3.186521739	b.d.l
B1	0.000919263	0.438060186	0.016017399	15.83129032	1.31173913	b.d.l
B3	0.000306536	0.507481447	0.030793976	13.95709677	1.655652174	b.d.l
S1	0.009382069	0.736772323	0.045900903	No Data	No Data	b.d.l
Inf	No Data	No Data	No Data	4.470741935	b.d.l	b.d.l
Eff	No Data	No Data	No Data	2.222951613	2.498695652	b.d.l

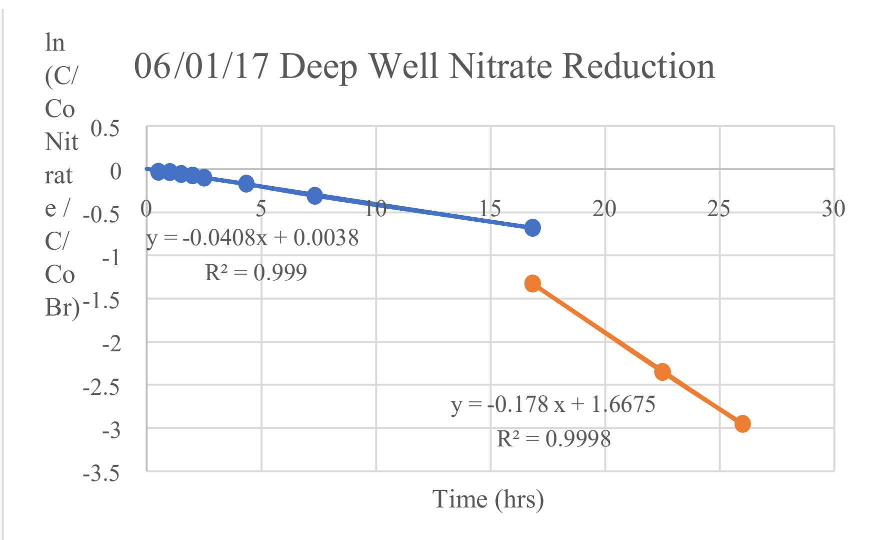
- **3e. Influent and Effluent Analyses** (Spring/Summer 2017 o *Time:* 30 hours**)**
  - Purpose: Determine average influent and effluent concentrations of nitrate, nitrite, and carbon in reactor.
  - Methods: Samples were taken and filtered at several different times. Samples were analyzed on both the IC and the TOC/TN machine to determine the concentrations of Total Nitrogen, nitrate, nitrite, Total Carbon, and Total Organic Carbon. Samples were taken over a 30-minute time-period to account for minute differences in flow rate
  - Results: Nitrate results were originally much higher than expected. But after using the 30-minute method, results were like the influent expected and sampling seemed to indicate that the 20mg/L nitrate-N was entering the system through the influent, with effluent nitrate-N at 10mg/L.
    - However, it seems that carbon concentrations were much higher than expected in the influent, with an average of 12mg NPOC/L in the influent when only 5mg C/L was expected. This may indicate the presence of organismal growth in the influent bags and tubing. Effluent concentrations were about 14 mg NPOC/L.

- *Overall Part III Results*
  - **Monitoring Well Tests:**
    - **Nitrate concentrations were higher for R2** in root-excluded bags than in root-exposed wells in March, April, and Summer Test
    - **Methane concentrations were** lower in root-excluded bags than in root-exposed wells on April, May, and Summer tests.
    - **CO2 concentrations were lower** in April, May, and Summer for root-excluded bags than in root-exposed wells
  - **Influent and Effluent Tests:**
    - Tests indicated nitrate in the effluent was about ½ of nitrate in the influent

Part IV. Research Experiment #2 (Comparing Water and Gas Chemistry Between Wetland Soil and Root-Excluded Bags

****In All tests B1 is a root-excluded bag well. M2, D3, and D1 are root-exposed wells****

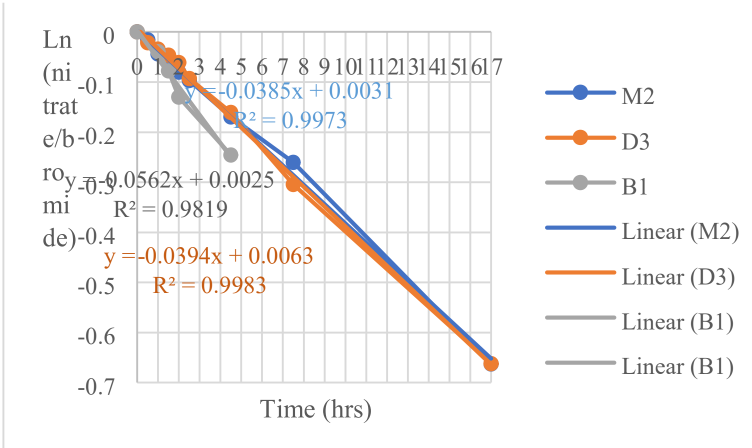
- **Push-Pull Test 1 (Nitrate-Only, 1 well) single well push pull test** (Spring/Summer 2017 o *Time:* 45 hours**)**
  - Purpose: Determine nitrate reduction rate in the reactor.
  - Hypothesis: Nitrate reduction will occur, consistent with those levels found in previous studies.
  - Method: This push-pull test was conducted over 26 hours, with an injection of nitrate and bromide 1 hour before time T=0.
  - Results:
    - As has been documented in literature, observed two distinct rates in nitrate reduction with increased rate by 3x after x hrs. Normalized with bromide, the reaction rates are:
      - 0.0408/hr for 0hrs-17hrs
      - 0.178/hr for 17-27 hrs
    - These results are consistent with previous literature data (Haggerty, Schroth, & Istok, 1998)

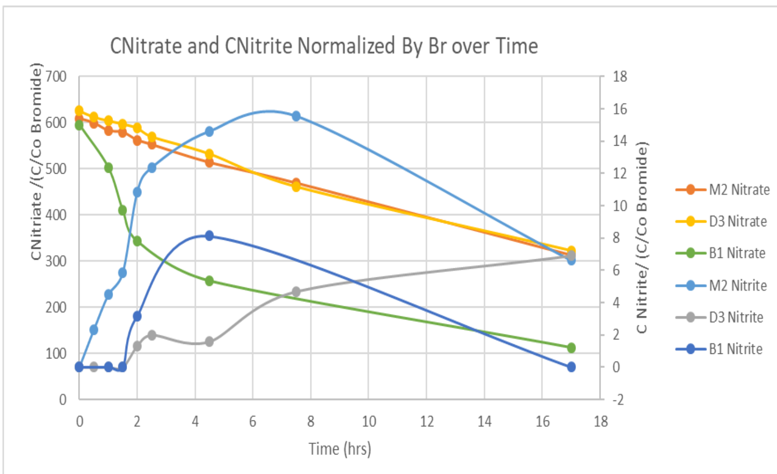


Part V: Research Experiment #3 (Push-Pull Test 2 Comparing Nitrate Reduction and Nitrous Oxide Formation in 2 Root-Exposed Wells and 1 Root Excluded Wells)

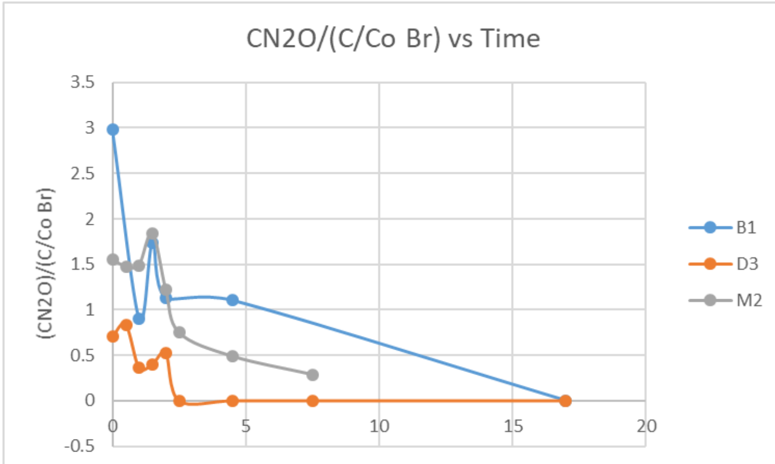
****In All tests B1 is a root-excluded bag well. M2, D3, and D1 are root-exposed wells****

- Purpose: This test was conducted to determine if there was a difference between nitrate reduction and n2o formation in wells exposed to roots vs wells that were not exposed to roots (bag)
- Hypothesis: Nitrate reduction will be different in root-exposed wells than in root-excluded bags.
- Method: This push-pull test tested nitrate reduction in 3 different wells, 1 deep well (D3), 1 mid-level well (M2), and one well contained within a bag and unexposed to roots (B1) with an injection of nitrate and bromide 1 hour before time T=0. Also measured nitrite to determine if there was any formation occurring. Nitrate and Nitrite concentrations were measured on the IC, while N2O concentrations were measured on the GC.
- 5a. Nitrate Reduction Results:
  - Normalized with bromide, reaction rates are:
    - 0.0385/hr for M2, half life= 3.92hrs
    - 0.0394/hr for D3, half-life= 3.95 hrs
    - 0.0562/hr for B1, half-life= 3.57 hrs
      - Results for M2 and D3 are consistent with literature results for nitrate reduction in planted constructed wetlands. Results for non-exposed well are indicate a shorter half-life for nitrate in areas not exposed to plants.
      - **Relative to the original objective, non-root bags had higher reduction rate than root-exposed monitoring wells.**





- <u>5b. $N2O$ Formation Results:</u>
    - Results for this data set also include nitrous oxide concentrations, which appear to be higher in the subsurface for the bag at later times than in deep and mid-level wells exposed to roots.



- ***Key Results from Part IV and V:***
    - Single Well Test Results:
        - Deep Well Closest to Influent: 0.0408/hr for D1
    - Multiple Well Test Results:
        - Mid-Level Well, Middle of Reactor: 0.0385/hr for M2, half life= 3.92hrs
        - Deep Well Furthest from Influent: 0.0394/hr for D3, half-life= 3.95 hrs
        - Root-Excluded Well: 0.0562/hr for B1, half-life= 3.57 hrs
    - Nitrate reduction rates were determined in root-exposed wells, in 3 different locations within reactor.
    - **Nitrate reduction rate is higher** in root-excluded bags
    - **N2O formation is higher** in root-excluded bag
-

Part VI: Research Experiments #4 and #5 (Paired Non-Acetylene with Nitrous Oxide and Acetylene with Nitrous Oxide Push-Pull Tests)

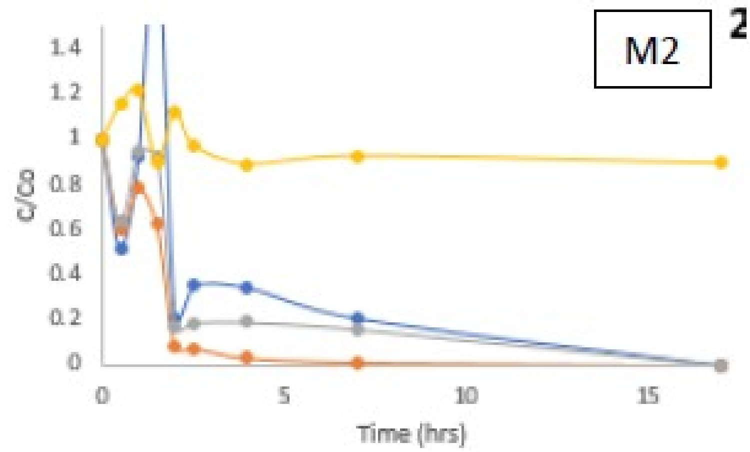
****In All tests B1 is a root-excluded bag well. M2, and D1 are root-exposed wells****

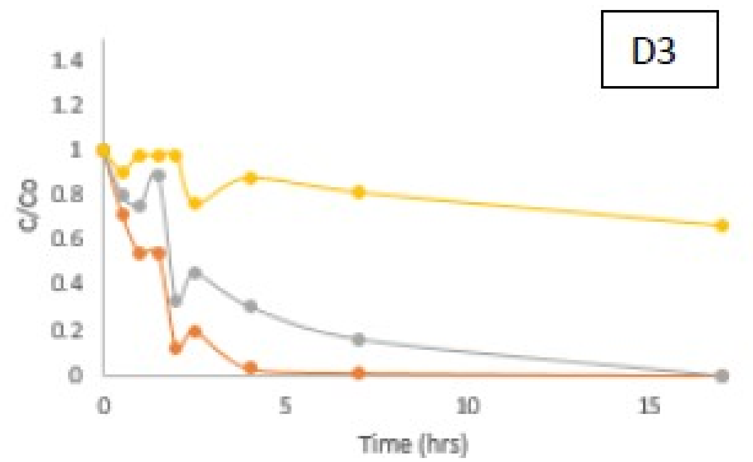
- **6a. Research Experiment #4 Push-Pull Tests 3&4 (N2O w/ and w/o Acetylene)** (Summer 2017 o *Time:* 80 hours)
    - Purpose: To determine if there was a difference in nitrous oxide emissions with wells exposed to roots vs wells that were not exposed to roots.
    - Hypothesis: Greater N2O reduction in root-exposed monitoring wells than in root-excluded bags when acetylene is present.

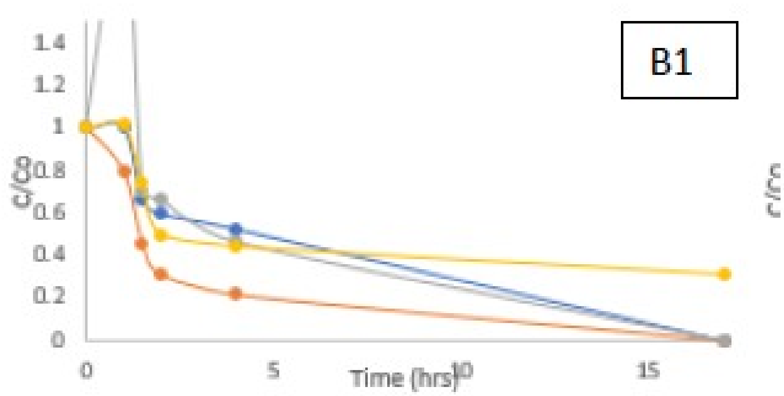
- Method: This push-pull test set was conducted as 2 separate 17-hour push-pull tests. The first test was conducted by injecting a hydrocarbon mix, N2O, SF6, and helium (where SF6 and Helium serve as gas tracers) into the subsurface at time T=0, the second test also added acetylene to the mix, an inhibitor of nitrous oxide to nitrogen gas.
- Results:
  - With Acetylene: When Acetylene is present, Reduction rates are higher for N2O in root-exposed sampling ports than in non-root exposed ports.
    - 0.65/hr D3
    - 0.63/hr M2
    - 0.17/hr B1
  - Without Acetylene: Higher N2O reduction rates in root-excluded bags than in root-exposed wells when acetylene is not present
    - 0.03/hr D3
    - 0.02/hr M2
    - 0.23/hr B1

Figures for Experiment with Acetylene

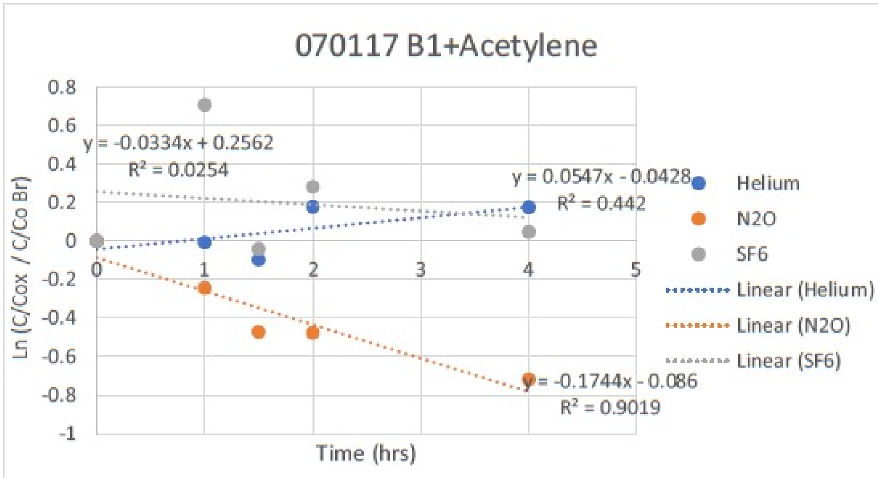
Time in hrs vs C/Co of helium (blue), N2O (orange), SF6 (grey), and bromide (yellow)

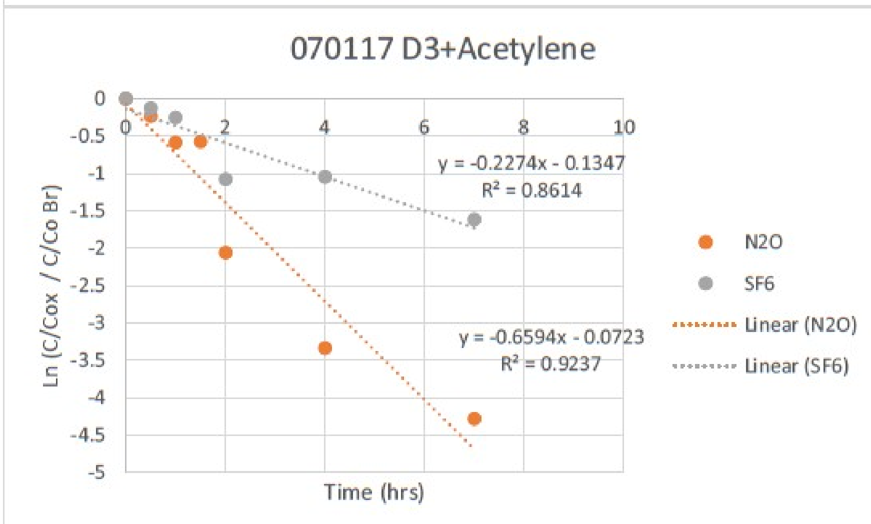


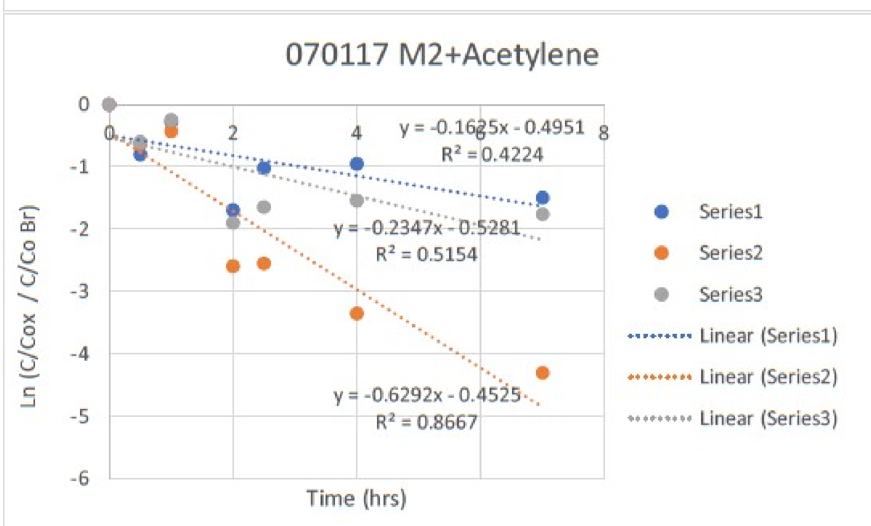


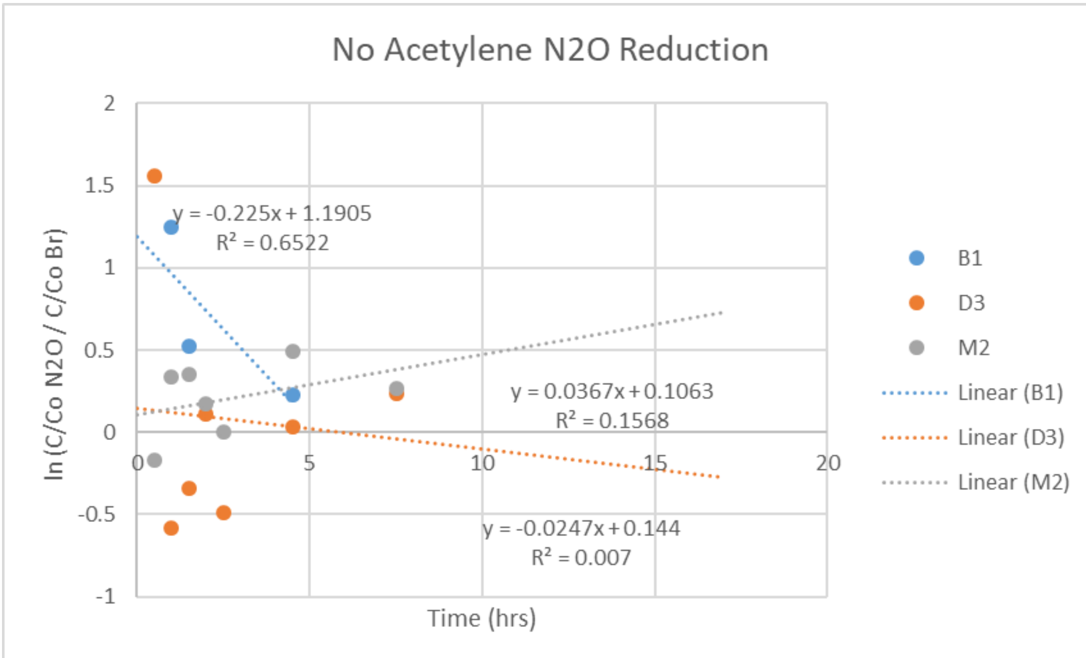


Reduction rates of helium, N_2O, and SF_6



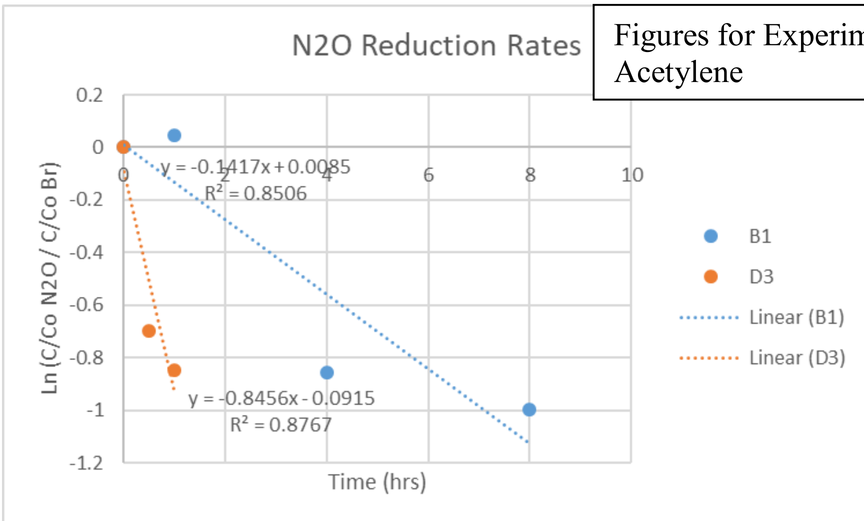


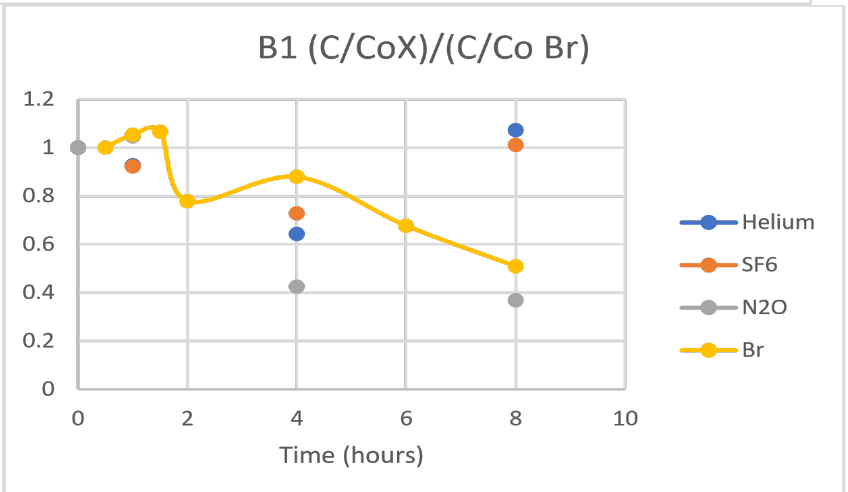


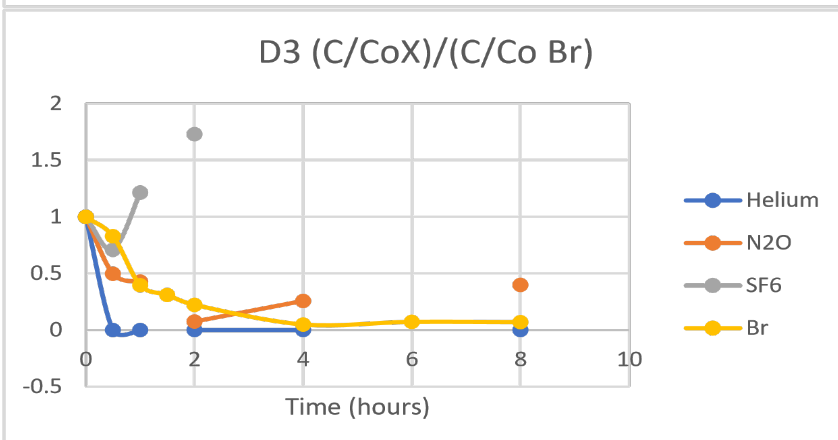


- **6b. Research Experiment #5 Push-Pull Tests 5&6 (N2O w/ and w/o Acetylene)** (Summer 2017 o *Time:* 80 hours**)**
    - Purpose: To determine if there was a difference in nitrous oxide emissions with wells exposed to roots vs wells that were not exposed to roots.
    - Hypothesis: Greater N2O reduction in root-exposed monitoring wells than in root-excluded bags when acetylene is present.
    - Method: This push-pull test set was conducted as 2 separate 8-hour push-pull tests. The first test was conducted by injecting a hydrocarbon mix, N2O, SF6, and helium into the subsurface at time T=0, the second test also added acetylene to the mix, an inhibitor of nitrous oxide to nitrogen gas.
    - Key Results:
        - Higher N2O reduction in root-exposed wells than in root-excluded bag when acetylene is present
        - Lower N2O reduction in root-exposed wells than in root-excluded bag when acetylene is not present

Figures for Experiment with Acetylene



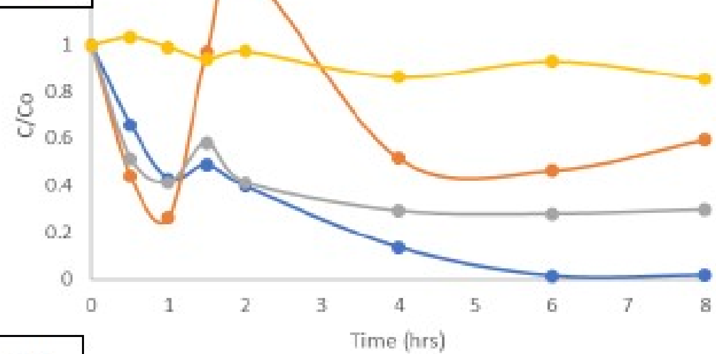




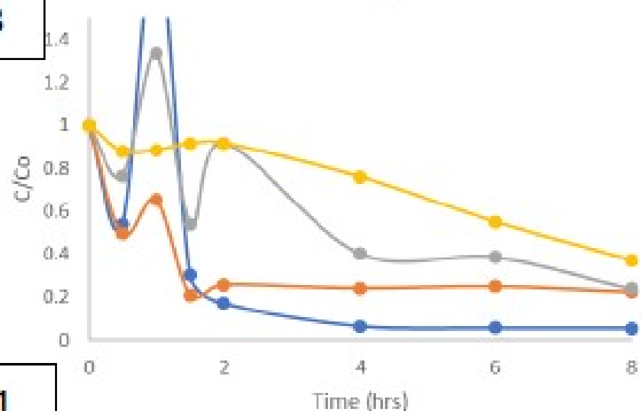
Figures for Experiment WITHOUT Acetylene

Time in hrs vs C/Co of helium (blue), N2O (orange), SF6 (grey), and bromide (yellow)

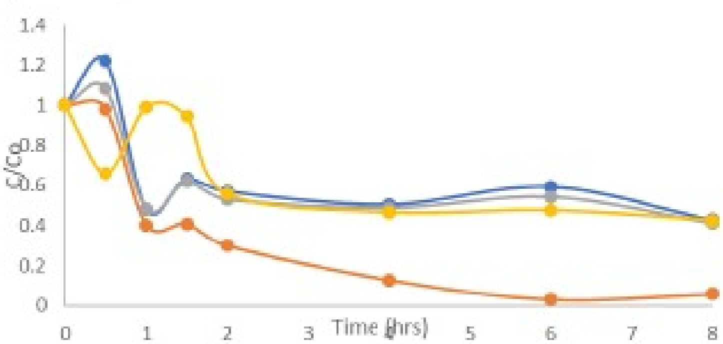
M2



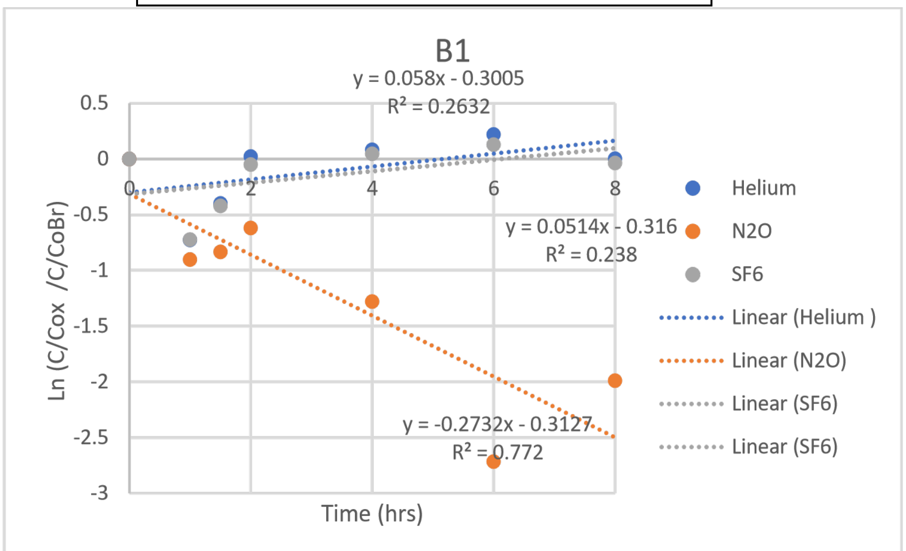
D3

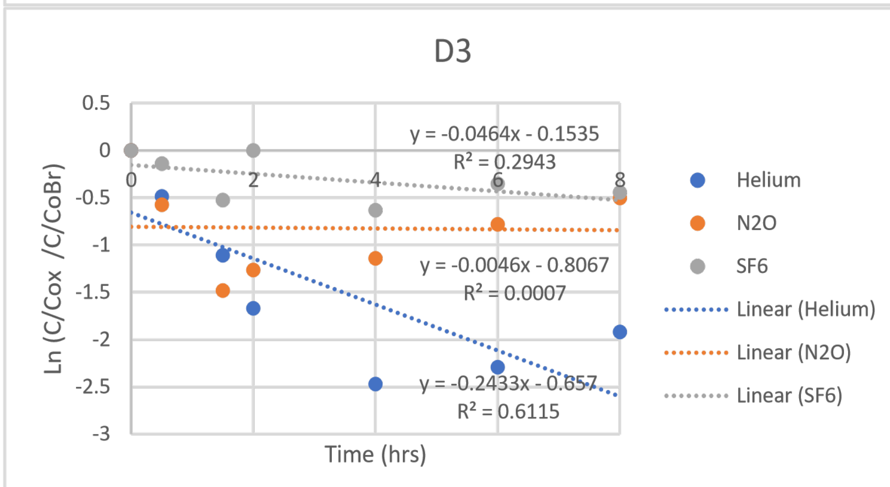


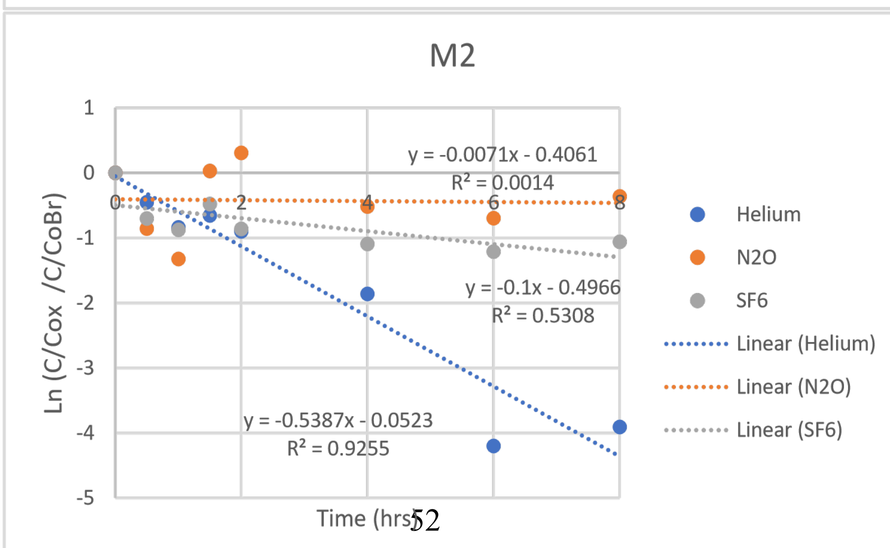
B1



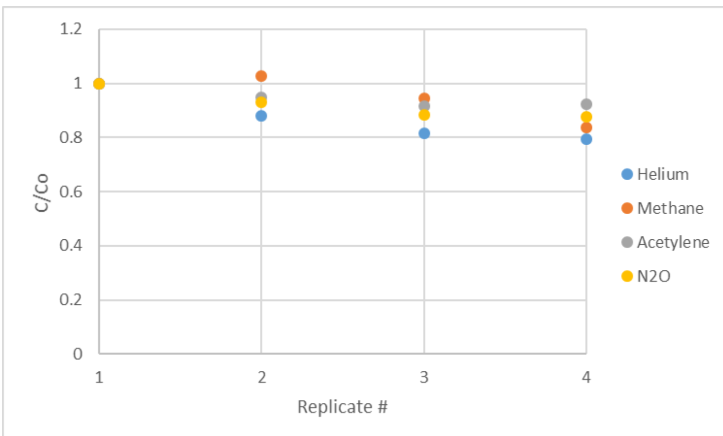
Reduction rates of helium, N2O, and SF6







- **6c. Injection Bag Tests** (Summer 2017 o *Time:* 6 hours**)**
  - <u>Purpose:</u> The purpose of this test was to determine if the concentration of the injection bag used for push-pull tests was high enough to be detected on the GC as well as to determine if those concentrations changed significantly after 1 hour.
  - <u>Hypothesis:</u> There will be some loss of gases in injection bags over time. This loss will probably be highest in helium because it is a very small molecule.
  - <u>Methods:</u> Gas bag bubbled with helium for 1 hour and methane, n2o and acetylene were added. 4 samples taken from gas bag and 3 samples taken immediately after preparation. 1 sample taken 1hr after preparation.
  - <u>Results:</u>
    - After 1 hour, all concentrations remained at least 80% of the original concentration, with helium and nitrous oxide as the most variable.



- ***Key Results from Part VI:***
  - Higher N2O reduction rates in root-excluded bags than in root-exposed wells when acetylene **is not** present
  - Lower N2O reduction rates in root-excluded bags than in root-exposed wells when acetylene **is** present
  - **Comparable and predictable rates of nitrous oxide lost in subsurface with root-excluded bags** with and without acetylene
    - <u>With Acetylene:</u>
      - 0.14/hr
      - 0.17/hr
    - <u>Without Acetylene:</u>
      - 0.27/hr
      - 0.23/hr

- When acetylene is not present, rates of nitrous oxide loss are not as predictable in root-exposed bags.
- Possible Explanations:
    - Acetylene blocks biological denitrification from N2O to N2
    - Thus, only physical transport via the roots or the soil is a possible mechanism when acetylene is present
    - When acetylene is not present, biological and physical transport methods are both present, which may explain less predictable rates for root-exposed wells

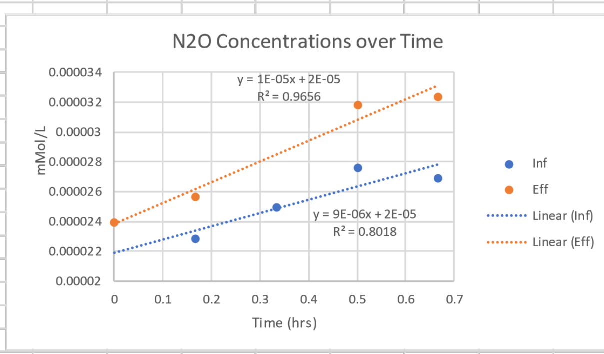
Part VII: Gas Flux Measurements (Summer 2017 o *Time:* 6 hours)

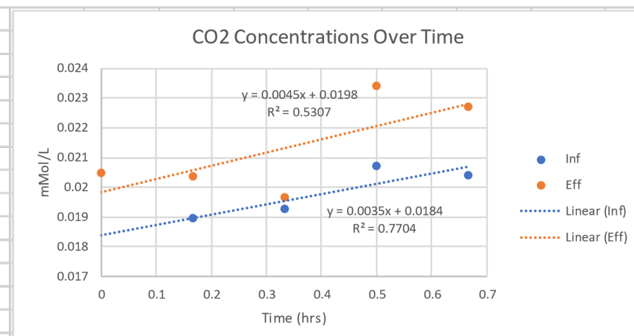
- Purpose: To determine if there is a gas flux present for N2O, CO2, or Methane in the system.
- Hypothesis: CO2, N2O, and methane will accumulate in the surface over time as roots move subsurface gases to the surface
- Methods: Gas flux measurements were taken over 40 minutes in 1 3ft chamber near the influent and 1 3ft chamber near the effluent.
- Results:
    - Highest fluxes for N2O, CO2, and Methane are near the influent
    - Data indicates a strong positive N2O flux in system.

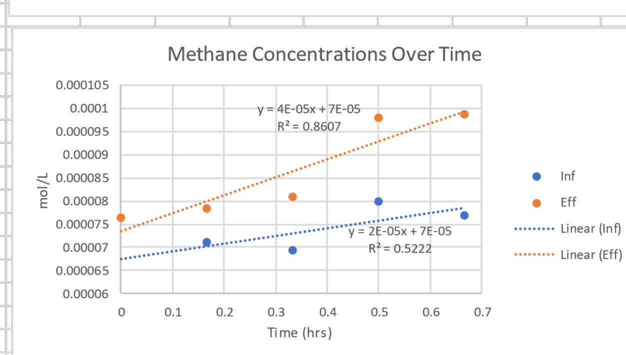
	Flux		
	N2O	CO2	Methane
Inf	9.84E-06	0.003828	2.19E-05
Eff	1.09E-05	0.004921	4.37E-05

$$\frac{(S\ \mathrm{mol\ L^{-1}\ hr^{-1}}) \cdot V\ \mathrm{L}}{A\ \mathrm{m^2}}$$

*Flux measured in these units







Part VIII: Preliminary Results Summary

- **Chemical Characterization of Reactors**
    - **Nitrate concentrations were higher for R2** in root-excluded bags than in root-exposed wells in March, April, and Summer Tests
    - **Methane concentrations were** lower in root-excluded bags than in root-exposed wells on April, May, and Summer tests.
    - **CO2 concentrations were lower** in April, May, and Summer for root-excluded bags than in root-exposed wells
- **Bromide Tracer Tests**
    - Preliminary Winter 2017 Test and Preliminary Fall 2017 tests indicate that in both cases, reactors behave like PFRs.
- **Influent and Effluent Tests**
    - Tests indicated nitrate in the effluent was about ½ of nitrate in the influent
- **Push-Pull Nitrate Tests**
        - <u>Single Well Test Results:</u>
            - Deep Well Closest to Influent: 0.0408/hr for D1
        - <u>Multiple Well Test Results:</u>
            - Mid-Level Well, Middle of Reactor: 0.0385/hr for M2, half life= 3.92hrs
            - Deep Well Furthest from Influent: 0.0394/hr for D3, half-life= 3.95 hrs
            - Root-Excluded Well: 0.0562/hr for B1, half-life= 3.57 hrs
    - Nitrate reduction rates were determined in root-exposed wells, in 3 different locations within reactor.
    - **Nitrate reduction rate is higher** in root-excluded bags
    - **N2O formation is higher** in root-excluded bag
- **Push-Pull N2O Tests**
    - Higher N2O reduction rates in root-excluded bags than in root-exposed wells when acetylene **is not** present
    - Lower N2O reduction rates in root-excluded bags than in root-exposed wells when acetylene **is** present
    - **Comparable and predictable rates of nitrous oxide lost in subsurface with root-excluded bags** with and without acetylene
        - <u>With Acetylene:</u>
            - 0.14/hr
            - 0.17/hr
        - <u>Without Acetylene:</u>
            - 0.27/hr

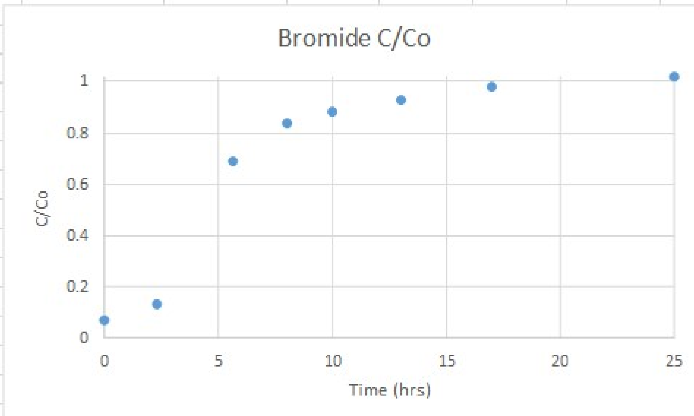
            - 0.23/hr
    - When acetylene is not present, rates of nitrous oxide loss are not as predictable in root-exposed bags.
    - Possible Explanations:
        - Acetylene blocks biological denitrification from N_2O to N_2
        - Thus, only physical transport via the roots or the soil is a possible mechanism when acetylene is present
        - When acetylene is not present, biological and physical transport methods are both present, which may explain less predictable rates for root-exposed wells
- **Gas Flux Measurements**
    - N_2O, CO_2, and Methane Fluxes were highest near the influent than the effluent
    - Strongest flux is a positive N_2O flux over time.

Part IX: Proposal and Setup for Fall Experiments

- **See Appendix 2 for Proposal for Fall 2017 Experiments**
- **9a. New Reactor Construction and Setup** (Fall 2017 o Time: 50 hours)
    - Purpose: Create a new reactor for use in Fall 2017 experiments.
    - Methods: Reactor was created with the same sampling regime as old reactors, however sampling ports were drilled through the side and reinforced with silicone to ensure reduced excess tubing. The reactor also had 3 influent ports and 3 effluent ports. Inoculum soil was collected from Beebe Lake at 10% by mass, and a ground leaf carbon amendment was created and added at 5% by mass. The remaining 85% of mass was added as multi-purpose Lowes sand. Mass was mixed and water was flown through the system at 30mL/min ▪ *Results:*


- **9b. Preliminary Bromide Tracer Test** (Fall 2017 o Time: 30 hours)
  - Purpose: Test conducted to characterize the flow pattern of the reactor
  - Methods: 5mM bromide solution run through system at 30mL/min over the course of 25 hours. Samples were taken intermittently throughout the 25-hour period.
  - Results: Appears to be a PFR

	Time (hrs)	C/Co
T0 825 7am	0	0.06823
T1 825 920am	2.333333	0.133952
T2 825 1240pm	5.6666663	0.692657
T3 825 3pm	7.999999633	0.839743
T4 825 5pm	9.999999633	0.882491
T5 825 8pm	12.99999963	0.92765
T6 826 12am	16.99999963	0.982205
T7 826 8am	24.99999963	1.017353



- **9c. In-Depth Bromide Tracer Test** (Fall 2017 o Time: 35 hours)
    - <u>Purpose:</u> Test conducted to characterize the flow pattern of the reactor
    - <u>Methods:</u> 5mM bromide solution run through system at 30mL/min over the course of 25 hours. Samples were taken intermittently at once every 30 min for the first 2.5 hours, once every 20 minutes for 2.5 hours to 7.5 hours, once every 30 minutes for 7.5-12 hours, and once every 3 hours for 12-18 hours, and a final time point at 25 hours. Final time point taken by undergraduate student. Bromide concentrations will be analyzed on the IC.
        - <u>Results:</u> To be analyze

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